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I. Willink, B. and Svensson, E. I. (2017) Intra- and intersexual differences in parasite resistance and female fitness tolerance in a polymorphic insect. *Proceedings of the Royal Society of London B: Biological Sciences* 284:20162407.1

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VI. Willink, B. and Svensson, E. I. (2018) Ecology and sexual conflict as macroevolutionary drivers of female-limited colour polymorphisms. *Manuscript*

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The descent of damselflies

and variation in relation to sex

Beatriz Willink Castro

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Abstract

Sexual conflict over mating shapes the interactions between males and females in many animals and is also responsible for dramatic adaptations in both sexes. In some species of pond damselflies (Odonata:Coeangrionidae), sexual conflict maintains discrete female-limited colour morphs over multiple generations and within populations. One of the female morphs is typically male-coloured and considered a male mimic. This is because their male-like appearance provides a frequency-dependent advantage against excessive male mating attempts. In this thesis, I investigate three major questions regarding the evolutionary consequences of this pervasive sexual conflict. First, how is phenotypic variation in ecological traits distributed among heritable female colour morphs? Second, how does sexual conflict shape phenotypic variation within the lifespan of females? Finally, how, where and why do female-limited morphs arise in the first place?

In the Common Bluetail damselfly (*Ischnura elegans*), female morphs differ in multiple phenotypic traits. My results uncover further phenotypic associations between the two most common morphs of the Common Bluetail in Sweden. One morph is more resistant to infections by parasitic mites, whereas the other is instead more tolerant. These morphs also differ in their developmental sensitivity to temperature, which in turn influences how morph frequencies are distributed across European populations. Moreover, my findings provide some insights as to how these profound phenotypic differences are produced over the course of adult development, and suggest that male mimics and non-mimics differ in the regulation of important developmental processes.

Females of the Common Bluetail undergo dramatic colour changes as they become sexually mature. My thesis shows that immature colour patterns in non-mimic female morphs reduce male pre-mating harassment, and may have evolved by co-opting male colour signals to be expressed as immature signals of reproductive unsuitability. These results suggest that female colour patterns might be highly evolutionarily labile. Yet, a large-scale phylogenetic framework is required to gain a full understanding of the macroevolutionary consequences of sexual conflict on the evolution of female-limited colour variation.

I inferred a multi-locus phylogeny for the damselfly superfamily Coeangrionoidea. I then used this phylogeny to show that female-limited colour polymorphisms have arisen repeatedly in this clade, and in association with ecological conditions that foster sexual conflict over mating. Finally, my results uncover a stark contrast between the consequences of sexual conflict at micro and macroevolutionary scales. While sexual conflict promotes diversity within populations by maintaining alternative female morphs, the presence of these morphs is also associated with increased extinction risk and a fast lineage turnover. Together, my results reveal how sexual conflict can influence the origin, distribution and loss of diversity.

Key words Sexual conflict, development, diversification, pleiotropy, correlational selection, damselfly

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“We are not here concerned with hopes or fears, only with truth as far as our reason permits us to discover it.”

Charles Darwin, *The Descent of Man and Selection in Relation to Sex*

Contents

List of Papers

I. Willink, B. and Svensson, E. I. (2017) Intra- and intersexual differences in parasite resistance and female fitness tolerance in a polymorphic insect. *Proceedings of the Royal Society of London B: Biological Sciences* 284:20162407.[1]

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Author Contributions

I. E.I.S. initiated the study and maintained the long-term population study, which provided the data. E.I.S. and B.W. participated in field work. B.W. compiled the data and designed and conducted the statistical analyses. B.W. wrote the manuscript with input from E.I.S.

II. E.I.S conceived the study with input from M.C.D and B.W. M.C.D and B.W. obtained the sequence data. C.W. assembled the trancriptome. B.W. performed the differential expression analyses and functional annotations with help from M.C.D. E.I.S. provided overall guidance throughout the work. B.W. wrote the manuscript with input from all authors.

III. The study was planned by E.I.S. and B.W. E.I.S. maintained the long-term population study, which provided the field data. B.W. designed the experiments and comparative analyses with help from E.I.S. B.W. and M.C.D. conducted the experiments. B.W. collected the molecular data, performed the statistical analyses and wrote the first draft of this paper. E.I.S. and M.C.D. contributed to the writing of the final manuscript.

IV. E.I.S. conceived the study, maintained the long-term population study, gathered and analysed the data on morph frequency variation across Europe. E.I.S., M.C.D. and B.W. collected the colour development and survival data. E.I.S. and L.L. performed the biogeographic analyses. E.I.S. analysed the colour development and survival data with input from B.W. B.W. performed the pre-reproductive selection analysis with input from E.I.S. E.I.S. wrote the manuscript with input from all authors.

V. E.I.S. and J.W. conceived the study and provided guidance throughout the work. E.I.S. and B.W. collected the field samples. B.W. collected the museum samples, performed the molecular laboratory work and designed and conducted the phylogenetic analyses. B.W. wrote the manuscript with input from E.I.S.

VI. E.I.S. and B.W. planned the study and collected the field data. B.W. compiled the literature data and designed and conducted the phylogenetic comparative analyses and discussed the results with E.I.S. B.W. wrote the manuscript with input from E.I.S.

Abstract

Sexual conflict over mating shapes the interactions between males and females in many animals and is also responsible for dramatic adaptations in both sexes. In some species of pond damselflies (Odonata:Coeangrionidae), sexual conflict maintains discrete female-limited colour morphs over multiple generations and within populations. One of the female morphs is typically male-coloured and considered a male mimic. This is because their male-like appearance provides a frequency-dependent advantage against excessive male mating attempts. In this thesis, I investigate three major questions regarding the evolutionary consequences of this pervasive sexual conflict. First, how is phenotypic variation in ecological traits distributed among heritable female colour morphs? Second, how does sexual conflict shape phenotypic variation within the lifespan of females? Finally, how, where and why do female-limited morphs arise in the first place?

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A day of collecting damselflies along the Rupununi river, Guyana. Photo E.I.S.

Popular Science Summary

We are lucky to live in a changing world, and with change comes variation. The immense variation in the external features of animals, and particularly their colour, fascinates scientists and non-scientists alike. In this thesis, I explore several aspects of why and how colour variation evolves in a charismatic group of insects. Pond damselflies are colourful, diurnal and visually driven organisms. Males use their vision to find females with whom they will attempt to mate. Females, however, would often fare better without excessive male attention, which reduces their fecundity. This fundamental conflict between the sexes arises because, as in many other animal species, damselfly females invest more time and energy in each of their offspring than males do, hence their reproductive success does not require access to many males. In contrast, it is in the best interest of males to mate with multiple females. My research has dealt with the consequences of this form of sexual conflict for the evolution of pervasive female diversification.

In some species of damselflies, females, but not males, occur in discrete colour morphs. These female morphs have remarkably different colour patterns and there are no intermediates. In fact, as far as we know, a single gene determines whether a female develops into one colour morph or another. My research shows that these female-limited colour polymorphisms have evolved repeatedly and independently over the nearly 40 million-year history of pond damselflies. Moreover, female colour morphs arise in damselflies under ecological conditions that promote conflict between females and males. This occurs, for instance, when many adult individuals are confined to a restricted breeding area and have a short time window of opportunities to mate.

One of the female morphs in pond damselflies is typically a male mimic, which avoids potentially harmful male-mating attempts due to its resemblance to males. A statistical reconstruction of the ancestral history of pond damselflies showed that when female-limited colour polymorphisms evolve, it is due to the origin of a new male-mimicking morph, in an ancestral species where males and females looked different from each other. This further suggests that sexual conflict is the major cause for the evolution of multiple female morphs. For females, however, the risks of unsought male persistence start early, before they are even capable of reproduction. The results of my thesis also suggest that male-like colour signals have evolved repeatedly among females that are not male mimics, but these females only display such colour patterns when they are sexually immature. These immature colour signals also discourage males from approaching females.

Are there any consequences of the evolution of female colour morphs, beyond the interactions between the sexes? This thesis, along with previous studies, also shows that female morphs differ in a variety of other characteristics that influence female survival and reproduction. My research provides evidence that the gene that controls morph determination influences many other genes during the course of female development. In one particular species, the Common Bluetail, these gene interactions may underlie the differentiation between male-mimics and non-mimics in the timing of several important developmental events. Moreover, these interactions have consequences for the performance of females in different external environments. I show that the two most common morphs in the Common Bluetail in Sweden, differ in their defence strategies against parasitic water mites. One morph does better at fending off the mites, whereas the other sees its reproduction nearly unaffected by mite infections.

If male-mimicking females can avoid some male harm with their appearance, one may ask, why are not all females mimicking males? The answer is that the benefits of male mimicry are reduced as the male mimics become more common in a population. Under such conditions, males might be less prone to fall for their deceit and alternative female morphs, which are now off-target, may thrive. This is why, within populations, sexual conflict can maintain female-limited colour variation over long periods of time, as the fates of the alternative morphs cycle through generations. However, over even longer time scales, sexual conflict may have very different consequences. I found that the evolution of multiple female morphs is also associated with a higher extinction risk. Sexual conflict can therefore have profound effects on many of the characteristics of animal species and influence the origin, maintenance and loss of their diversity.

Resumen de Ciencia Popular

Tenemos suerte de vivir en un mundo cambiante, y con los cambios viene la variación. La inmensa variación en las características externas de los animales, y en particular su color, fascinan tanto a los científicos como a los que no lo son. En esta tesis exploro varios aspectos sobre por qué y cómo evoluciona la variación en el color de un grupo carismático de insectos. Las libélulas de charco son organismos coloridos, diurnos y que se orientan con visualmente. Los machos usan su visión para encontrar hembras, con las que tratarán de aparearse. A las hembras, en cambio, les iría mejor sin la excesiva atención de los machos, la cual reduce su fecundidad. Este conflicto fundamental entre los sexos se debe a que, como en muchos otros animales, las libélulas hembra invierten más tiempo y energía en su progenie, por lo que su éxito reproductivo no depende de tener acceso a muchos machos. Los machos, al contrario, se benefician al aparearse con varias hembras. Mi investigación ha tratado con las consecuencias de este tipo de conflicto sexual sobre la evolución de diversidad prominente entre hembras.

En algunas especies de libélulas, las hembras, pero no los machos, muestran morfotipos de color discretos. Estos morfotipos en hembras tienen patrones de color marcadamente distintos, y no existen intermedios. De hecho, hasta donde sabemos, un sólo gen determina si una hembra desarrolla un morfotipo u otro. Mis resultados muestran que estos polimorfismos de color limitados a hembras han evolucionado de manera repetida e independiente a lo largo de los casi 40 millones de años de la historia de las libélulas de charco. Aún más, los morfotipos de color en hembras se originan en condiciones ecológicas favorables a producir conflictos entre hembras y machos. Esto ocurre, por ejemplo, cuando muchos adultos se ven obligados a limitar su actividad reproductiva a áreas restringidas y periodos cortos.

En estás libélulas, las hembras de uno de los morfitpos son imitadoras de machos, lo que les permite evitar un costoso exceso de intentos de apareamiento por parte de los machos. Una reconstrucción estadística de la historia ancestral de las libélulas de charco demostró que cuando los polimorfismos de color en hembras evolucionan, lo hacen por medio del origen del morfotipo de imitadoras de machos, en una especie ancestral donde hembras y macho se ven diferentes. Esto también sugiere que el conflicto sexual es la principal causa de la evolución de distintos morfotipos en hembras. Para las hembras, sin embargo, los riesgos de la persistencia desfavorable de los machos empiezan temprano, aún antes de que sean capaces de reproducirse. Los resultados de mi tesis sugieren además que rasgos de color similares a los de machos han evolucionado en hembras que no son imitadoras de machos, pero estas hembras sólo muestran dichos rasgos mientras son sexualmente inmaduras. Estas señales de inmadurez por medio de color también desalientan a los machos de acercarse a las hembras.

¿Existen otras consecuencias de la evolución de los morfotipos de color en hembras, más allá de las interacciones con los machos? Esta tesis, junto con estudios previos, también demuestra que los morfotipos en hembras difieren en varias otras características, las cuales afectan la supervivencia y reproducción de las hembras. Mi investigación demuestra que el gen que controla la determinación de los morfotipos de color, influencia la actividad de muchos otros genes durante el desarrollo de las hembras. En una especie en particular, la cola azul común, estas interacciones dirigen la diferenciación entre los morfotipos de imitadoras de machos y hembras distintas de machos, en cuanto al momento en que se dan eventos de desarrollo importantes. Tan es así, que estas interacciones entre genes tienen consecuencias sobre el desempeño de las hembras en diferentes ambientes externos. Aquí demuestro que los dos morfotipos más comunes de la cola azul común en Suecia difieren en sus estrategias de defensa contra ácaros parasíticos. Uno de los morfotipos es mejor en impedir infecciones, mientras que para el otro la reproducción es prácticamente insensible a las infecciones.

Si las hembras imitadoras de machos pueden evitar el acoso por machos dada su apariencia, uno podría preguntarse, ¿por qué es que no todas las hembras imitan a los machos? La respuesta yace en que los beneficios de parecerse a un macho se reducen conforme las imitadoras se vuelven más comunes en la población. Bajo estas circunstancias, los machos serían menos sensibles a ser engañados, y los otros morfotipos, que ahora son menos buscados por machos, tendrían mayor éxito. Esta es la razón por la que, dentro de poblaciones, el conflicto sexual puede mantener la existencia de los polimorfismos de color en hembras, ya que la suerte de los diferentes morfotipos fluctúa a través de las generaciones. Sin embargo, a lo largo de periodos aún más extensos, el conflicto sexual puede tener consecuencias muy diferentes. He encontrado que la evolución de distintos morfotipos en hembras también se correlaciona con un mayor riesgo de extinción. Por lo tanto, el conflicto sexual puede tener efectos profundos sobre muchas características de especies animales y al mismo tiempo influenciar en ellas el origen, mantenimiento y pérdida de diversidad.

Populärvetenskaplig Sammanfattning

Vi har lyckan att leva i en föränderlig värld, och med förändringar följer variation. Den stora variationen i yttre egenskaper hos djur, och i synnerhet deras färger, fascinerar både forskare och lekmän. I den här avhandlingen utforskar jag flera aspekter på varför, och hur, färgvariation evolverar hos en karismatisk insektsgrupp. Dammflicksländor är färgglada, dagaktiva och visuellt drivna organismer. Hannarna använder sin syn för att finna honor som de försöker para sig med. Honorna däremot, skulle klara sig bättre utan överdrivet många parningsförsök från hannarna som minskar deras fortplantningsframgång och äggläggningstakt. Denna fundamentala konflikt mellan könen uppstår eftersom, i likhet med honor hos många andra djurarter, investerar flicksländehonor mer tid och energi i var och en av sina avkommor än vad hannarna gör, och deras reproduktionsframgång kräver därför inte tillgång till många hannar. För hannarna är det däremot reproduktivt fördelaktigt att para sig med många honor. Min forskning har fokuserat på konsekvenserna som denna typ av konflikt mellan könen får för evolutionen av omfattande honlig differentiering.

Hos vissa arter av flicksländor förekommer det diskreta färgmorfer hos honorna, men inte hos hannarna. Dessa honliga morfer har anmärkningsvärt skilda färgmönster och det förekommer inga mellanformer. Så långt vi vet så är det en enda gen som bestämmer om en hona utvecklas till en färgmorf eller en annan. Min forskning visar att dessa honliga färgpolymorfier har utvecklats flera gånger oberoende av varandra under dammflicksländornas, nästan 40 miljoner år långa, evolutionära historia. Vidare så uppstår honliga färgmorfer hos flicksländor i ekologiska miljöer som är associerade med konflikter mellan hannar och honor. Till exempel när många könsmogna individer förekommer inom ett begränsat reproduktionsområde samtidigt och har ett kort tidsfönster att para sig.

En av honmorferna hos dammflicksländorna är vanligtvis en hanlik ”härmare” som undviker potentiellt skadliga parningsförsök genom att likna en hanne. En statistisk analys av dammflicksländornas evolutionära historia visade att när honliga färgpolymorfier utvecklas så är det genom uppkomsten av en ny hanlik och han-härmande morf hos arter där hannar och honor ser olika ut. Detta stärker ytterligare hypotesen att könskonflikter är den huvudsakliga drivkraften bakom evolutionen av flera olika honliga färgmorfer. För honorna börjar emellertid risken för hanliga parningstrakasserier tidigt, innan de ens är könsmogna. Resultaten i min avhandling visar vidare att hanlika färgsignaler har evolverat flera gånger bland honor som inte är hanlika ”härmare”, och att dessa honor endast uttrycker sådana signaler innan de är könsmogna. Dessa juvenila färgsignaler får hannarna att undvika honorna.

Finns det några konsekvenserna av evolutionen av honliga färgmorfer, utanför interaktioner mellan könen? Den här avhandlingen, i likhet med tidigare studier, visar att honmorferna skiljer sig åt i ett flertal olika egenskaper som påverkar honornas överlevnad och reproduktion. Min forskning visar att genen som styr morfutvecklingen interagerar med många andra gener under honornas utveckling. I synnerhet hos den stora kustflicksländan verkar dessa geninteraktioner påverka differentieringen mellan de hanlika honorna (”härmarna”) och de andra honmorferna vad gäller när flera viktiga steg i utvecklingen sker. Vidare så får dessa geninteraktioner konsekvenser för honor i olika miljöer och under olika ekologiska förhållanden. Jag visar att de två vanligaste honmorferna hos den stora kustflicksländan i Sverige skiljer sig åt i sina försvarsstrategier mot parasitiska vattenlöss. En av honmorferna är bättre på att försvara sig mot dessa vattenlöss, medan den andra morfens reproduktion knappast påverkas av infektioner från dessa parasiter.

Om hanlika honor lyckas undvika en del av kostnaderna för hanliga parningsförsök genom sitt utseende, så kan man fråga sig varför inte alla honor försöker likna hannar. Svaret är att fördelarna med att härma hannar minskar när dessa hanlika honor blir alltför vanliga i populationen. Under sådana förhållanden skulle hannarna genomskåda ”härmarna” och de andra honliga färgmorferna skulle klarar sig bättre. Därför kommer könskonflikter inom populationerna att bibehålla den honliga färgvariationen över långa tidsperioder och de olika morferna kommer att skifta i sina frekvenser mellan generationerna. Över ännu längre tidsskalor så kan dock könskonflikterna få andra konsekvenser. Jag visar i min avhandling att evolutionen av flera honmorfer också kan leda till förhöjd utdöenderisk. Könskonflikter kan därför få stora konsekvenser på många egenskaper hos olika djurarter och kan styra uppkomsten, bibehållandet och förlusten av variation.

Glossary[2]

Adaptation	The evolutionary process in which fitness increases as a product of natural selection. Also the phenotypic state resulting from that process
Allele	One of two or more alternative forms of the same gene
Clade	A group of taxa comprising an ancestor and all its descendants
Gene	A heritable unit encoded as DNA
Genetic drift	Changes due to chance in the allele frequencies of a population
Genome	All genes in an organism
Evolution	Change in the frequency of alleles in a population between generations
Fitness	The contribution of a particular genotype to the population in the next generation
Locus	*Plural:* loci. The physical position of a gene in the genome
Macroevolution	speciation, extinction and the persistence of species and higher taxa
Natural selection	The process of differential survival or reproduction when trait variation influences fitness
Phenotype	All characteristics of an individual that result from the interaction of its genes and the environment
Phylogeny	A hypothetical relationship between groups of species, genes or higher taxa, describing their shared and unique ancestry
Pleiotropy	Multiple phenotypic effects of a single gene
Recombination	When chromosomes exchange genes prior to halving the number of chromosomes in formation of eggs and sperm
Sexual selection	Variation in fitness that arises due to differential mating success, including pairing and fertilization
Taxon	*Plural:* taxa. A group of organisms treated as a unit by taxonomists, such as a species, genus, family, etc.
Trade-off	A situation that arises when two traits cannot be simultaneously optimized or a single trait cannot be simultaneously optimized for two different contexts
Trait	Any character or feature of an organism

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[2] The definitions of key biological concepts are not static. Here I use definitions, often simplified, that are consistent with large bodies of scientific literature and are convenient for understanding the specific issues addressed in this thesis.

Introduction

Evolutionary biology aims to understand how variation originates, is maintained and is distributed across living organisms. Because **phenotypes** are the targets of **selection** (West-Eberhard 1989; Mayr 1997), evolutionary ecologists are interested in how phenotypic differences arise from environmental differences. Two questions that may seem trivial at the outset are, in my perspective, at the core of this field. First, why do organisms differ at all in their phenotypes? And second, why do we currently observe certain phenotypic differences, and not others? These utterly general questions underlie every study in the present thesis, which deals with different aspects and scales of phenotypic variation. Specifically, I studied phenotypic variation that is maintained among genetic clusters within populations (Papers **I** and **II**), phenotypic variation that is produced over the course of development within individuals (Papers **III** and **IV**), and phenotypic variation that is originated throughout **macroevolution** (Papers **IV**, **V** and **VI**).

Phenotypes are the products of interactions between linear genetic sequences and the internal and external environments in which **genes** are potentially expressed (Lewontin 1974). From a single gene's perspective, the environment includes everything from other **loci**, their translated products and regulatory influences, to the ecological and social selective regimes which their bearing organisms encounter. Therefore, phenotypes are necessarily multidimensional and hence challenge our ability to describe them in a way that is both meaningful and practical. Consequently, evolutionary biologists must often prioritize which axes of variation to investigate (Houle et al. 2010). In this thesis, I focus on variation in or related to sex-limited colour patterns, as a means to reduce dimensional complexity and yet address important questions about phenotypic **evolution**. I also narrow down my study systems, in some cases to a single species and in others to larger **clades** of damselflies, which are diurnal insects characterised by remarkable variation in colour patterns (Fig. 1).

Colour **traits** are both fascinating and often practical to study. As we are ourselves visually-driven organisms, the aesthetic appeal of animal colour patterns is sufficient motivation to investigate the processes that have driven their evolutionary history. It therefore comes as no surprise that some of the **adaptive** hypotheses to explain variation in colour traits are about as old as evolutionary biology (Darwin 1874; Wallace 1889; Poulton 1890). Heritable and discrete colour morphs are also suitable for reducing the causal axes of variation, as such colour variants are often governed by one or a few linked loci with **pleiotropic** effects over suites of correlated traits (Mckinnon and Pierotti 2010). In these systems, colour can function as a visual marker of ecologically-relevant genetic variation, and colour-polymorphic species can be used to draw inferences about general processes driving phenotypic evolution (Svensson 2017).

The aim of this thesis is to ask specific questions about how discrete phenotypic variation is maintained and is distributed among female-limited colour morphs in damselflies and why this form of variation arises in certain lineages and is expressed at certain times. I addressed these questions while striving to discern conceptual links between the complementary perspectives of ecology, history and development, which are all required to understand phenotypic evolution. Thus, while most PhD students probably become increasingly specialized during the course of their studies, I have attempted to maintain a ‘rather blurred vision’[3]. This goal demanded a toolbox of diverse approaches, including classic population and field biology, behavioural experiments, transcriptomics and phylogenetic and comparative methods. In the following pages, I will take a closer look at some of the mechanisms that produce and maintain phenotypic variation. I will then show how applying this diverse set of tools to studying sex-limited colour morphs can help us further understand such mechanisms and their joint effects shaping phenotypic diversity.

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[3] Robert MacArthur (1968) referred to the importance of diverse perspectives among ecologists, with some elucidating the causes for ecological differences among organisms and others, ‘with rather blurred vision’, finding repeated patterns among them.

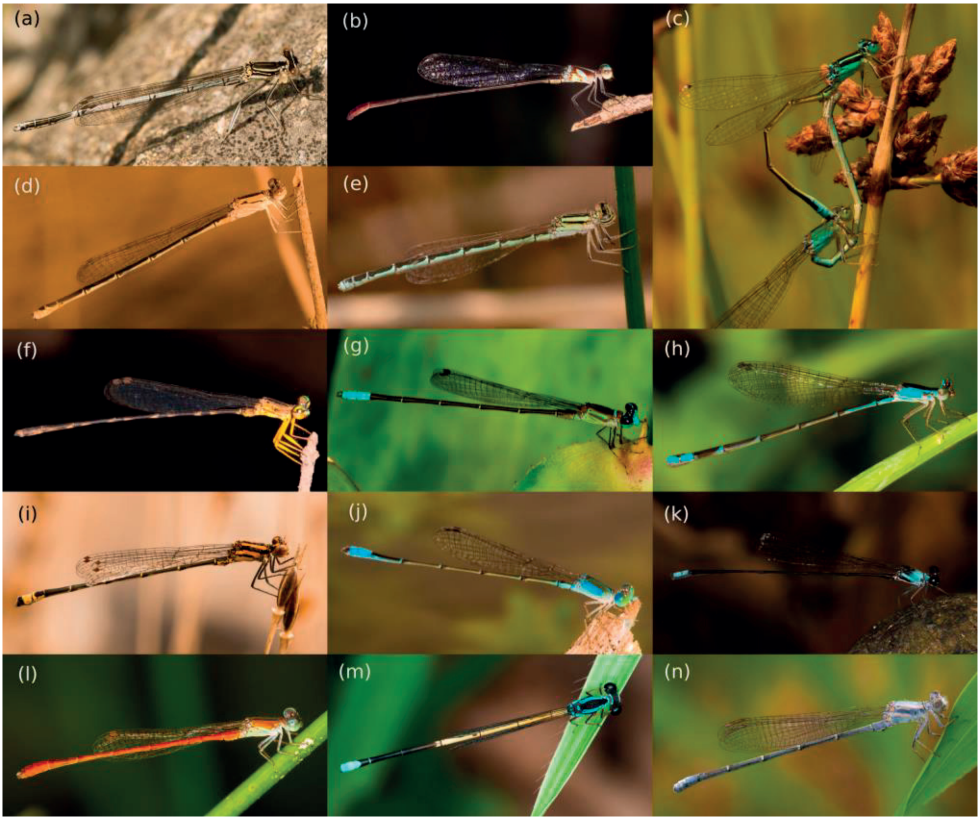

Figure 1. The colour patterns in damselflies of the superfamily Coenagrionoidea are extraordinarily diverse. Here are some representative species photographed in the field by E.I.S. **(a)** *Platycnemis pennipes*, female, **(b)** *Phoenicagrion flammeum*, male, **(c)** *Ischnura pumilio*, mating couple, **(d)** *Pseudagrion nubicum*, female, **(e)** *Acanthagrion minutum*, female, **(f)** *Copera congolensis*, male, **(g)** *Leptagrion elongatum*, male, **(h)** *Nehalennia minuta*, female, **(i)** *Acanthagrion adustustum*, male, **(j)** *Telebasis demarara*, female, **(k)** *Elattoneura balli*, male, **(l)** *Telebasis simulata*, male, **(m)** *Minagrion riberoi*, male, **(h)** *Argia moesta*, male.

Background

Evolutionary biologists recognize that selection is a creative force and it does more than removing unfit **alleles** from a population (Darwin 1859; Fisher 1934). Selection acts on gene combinations, which carry the signatures of past selection, as they are expressed in particular environments. Selection thus influences the additive effects of genes, their co-distribution among individual **genomes**, their interactions with each other and their responsiveness to the environment. All these outcomes of selection shape phenotypic variation. However, it is also clear that adaptation occurs within limits, and we owe to its imperfection that no organism lives forever, is instantly mature and produces infinite offspring (Law 1979). Here, I first review some of the selective mechanisms that mold phenotypic variation in colour traits involved in inter-sexual interactions. Then, I describe how the developmental and historical contexts in which such mechanisms operate are important for understanding their outcome.

Classic explanations for colourful traits

Darwin was first to point out that females[4] often prefer to mate with males displaying characters so extreme that may even be deleterious to their own survival (Darwin 1874). These characters typically include conspicuous colour patterns which are preferred by females but also more easily detected by predators (e.g. Endler 1978). It was difficult for Darwin, to imagine how such seemingly harmful preferences might evolve in females and why the preferred ‘modifications’ were not inherited to both sexes. With a few exceptions (Fisher 1930), however, these fundamental questions were largely overlooked until well within the 20th century, when Darwin’s uncomfortable proposition that females could exert a sexual choice was revived (Prum 2017). In spite of this delay,

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[4] Sexual selection predicts that competition for mating opportunities will arise among members of the sex more readily available for mating. Males are typically the surplus sex, due to their low investment in offspring provisioning and survival compared to females. The resulting male bias in the operational sex ratio provides a greater opportunity for female choice of mating partners than *vice versa* (Trivers 1972).

evolutionary biologists have hotly debated for the last half of a century why such female mating preferences evolve, particularly in cases where males provide nothing but genetic material to their offspring.

Traditionally, the two camps of this debate have been divided over whether the evolution of female preferences for male traits that reduce their survival ultimately requires a non-sexual advantage. For the adaptive camp, female preferences for costly display traits evolve because it is only the males of superior genetic quality who can afford to express viability-impairing ornamentation (Zahavi 1975). Females would then benefit by passing on these *good genes* to both daughters and sons who will have a better chance at survival.

While verbally appealing, this model is possibly of limited interest for explaining instances of dramatic phenotypic diversification of traits used in inter-sexual displays. The *good genes* model is based upon a reliable correlation between male quality and the elaboration of display traits, in which high quality males pay proportionally lower survival costs for their conspicuous ornamentation (Grafen 1990). Thus, for female preferences to become advantageous, all previous variation in a display trait that is independent of male genetic quality should be removed. Once this correlation is established, females will also exert directional selection for male viability through their choice of costly displays, unless the natural selection costs of the preference itself are prohibitive (Fuller et al. 2005). By doing so, genetic variation for the display trait will be further reduced. Therefore, the mechanisms that impose honesty in inter-sexual signals also limit their capacity to evolve (Prum 2010). In terms of the evolution of phenotypic diversity, the good genes model throws away the baby with the bath water.

One possible exception, which has been vastly used for providing empirical support for the good genes model, is dependent on host-parasite coevolution (Hamilton and Zuk 1982). As parasite virulence increases it selects for more resistant hosts. Once the majority of hosts are resistant, selection for resistance is relaxed causing virulence to rise again. The alleles that confer higher survival, those that provide resistance against current parasites or those that spare the cost of resistance when virulence is low, will vary across these cycles, renewing genetic variation for viability and therefore genetic variation for the display trait.

The nonadaptive camp on the sexual selection debate argues that the evolution of female preferences should be first addressed under a simpler model which does not require any extrinsic selective pressures (Prum 2010). The most vigorous males may be the ones that express the traits preferred by females, without the preference arising *because* the trait is condition dependent (Kirkpatrick 1987). Male traits may indicate nothing about their genetic quality and yet be driven away from their naturally selected optima by female choice.

This is shown by a model initially conceptualized by Fisher (1930) and then worked out independently by Lande (1981) and Kirkpatrick (1982). The L-K model (after Lande and Kirkpatrick) is initiated by only genetic variation in both the male trait and in the female preference. Males with trait values that match the mean female preference will be at an advantage. By mating with these males, females will indirectly select on their own preferences, as they favour the mothers of males who made a similar choice as themselves. This self-reinforcing process would inevitably lead to the genetic correlation between the alleles coding for display traits and the alleles coding for matching preferences.

Under the L-K model, natural selection acting against exaggeration of a display trait leads to a line of equilibrium between the two forces (Kirkpatrick and Ryan 1991; Fig. 2). Whether the equilibrium is stable or unstable depends on the amount of genetic variation in the display trait relative to the strength of the genetic correlation between preferences and traits (Lande 1981). Unstable equilibria represent a scenario in which the preference-trait correlation is so strong, that male traits continually evolve to match ever-demanding female preferences in an endless cycle. In nature, unstable equilibria, also known as the *Fisherian runaway* process, might be short-lived and halted as soon as female preferences are no longer cost-free. In the stable equilibrium, mean preferences and traits are attracted to the equilibrium line in Figure 2. However, small deviations due to **genetic drift** can result in substantial evolutionary change, as the population might arrive to a new equilibrium state along the line rather than return to the previous one (Lande 1981). This is the property that makes the L-K model, even at stable equlibrium, so amenable to the evolution of extensive phenotypic diversification by sexual selection.

In some circumstances, a female preference for conspicuous male colours may be an indirect effect of natural selection for signal efficacy (Endler and Basolo 1998). This situation in which natural selection penalizes female preferences for males that are difficult to detect or provide little sensory stimulation is known as *sensory drive*. Sensory drive can kick-start a L-K process by providing initial genetic variation in female preferences, for instance, after an ecological change that affects the visual environment. Sensory drive could also halt a L-K scenario that has driven female preferences far from their naturally selected optima. In many realistic scenarios, sensory drive and L-K conditions probably operate in tandem to determine the level of conspicuousness and the specific colour combinations that are selected in males.

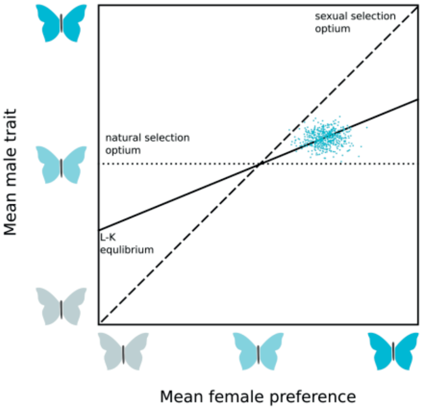

Figure 2. L-K equilibrium for the evolution of male display traits and corresponding female preferences. The equilibrium line reflects the balance of natural and sexual selection acting on male display traits. Populations can evolve rapidly along this line if they are displaced by drift. Based on Kirkpatrick and Ryan (1991).

Male colour patterns in polygynous species, in which males compete against each other and females exert their sexual preferences, are often extraordinarily variable among lineages (e.g. Dale et al. 2015). I believe that a substantial fraction of this outstanding variation is probably accounted for by the coevolution of male colour patterns and female preferences in somewhat arbitrary directions. However, as will be seen, the descent of damselflies is characterised by vast variation in mating systems, including many instances in which female preferences are not exerted so freely. In damselflies, this has led to remarkable colour variation within females, as well as between the sexes. To understand how such variation could evolve, I will turn to general mechanisms that can maintain discrete alternative phenotypes within species, including sex-limited colour morphs.

A rare advantage maintains variation

One of the processes that can maintain discrete and heritable phenotypic variation is negative frequency-dependent selection (NFDS). Frequency-dependent selection encompasses all instances in which the relative **fitness** associated with a particular trait depends on the frequency of each phenotype, in populations where two or more different phenotypes co-occur (Sinervo and Calsbeek 2006). NFDS occurs when a relatively rare phenotype thrives because its fitness is increased by interacting with individuals that are different from itself. Antagonistic interactions such as competition, predation and sexual conflict can often be negative frequency-dependent.

When individuals compete for mating opportunities or resources, one tactic usually involves territorial and aggressive behaviours towards competitors, at a cost of higher sensitivity to its own frequency (Svensson et al. 2001; Pryke et al. 2007; Kokko et al. 2014). Although territorial individuals are more likely to win contests against non-territorial individuals, they are also more likely to engage in contests with each other, and would therefore become more physiologically stressed in socially competitive environments (Svensson et al. 2001; Pryke et al. 2007) Aggressive competitors thus face a **trade-off** between high fitness if they are relatively rare and their rivals are easily subdued, and low fitness if they are common, and constantly engaged in strenuous fights. Other mechanisms of NFDS on competitive strategies include traits such as male cuckoldry and other life-history strategies, which have diminishing returns as their frequency and/or density increases (Gross 1991; Sinervo et al. 2000a).

Visually-oriented predators often have to actively search for prey displaying alternative phenotypes (Allen and Greenwood 1988). For these predators, a sensory limit on the rate of information processing is thought to underlie the evolution of transitional attention biases known as search images (Dukas and Kamil 2000; Dukas and Kamil 2001). Because searching for prey can be a perceptually arduous task and different prey types make competing attentional demands, predators that can switch their search image for the most common prey type will have greater performance at any given time (Merilaita and Ruxton 2009). Flexible search image formation therefore results in a disproportionally high detection of common prey phenotypes, resulting in NFDS on prey visual traits (Bond 2007). Accordingly, experimental studies have shown a survival advantage of rare colour patterns in prey, which may be linked to the maintenance of prey colour polymorphisms across several generations (Bond and Kamil 2002; Olendorf et al. 2006; Fitzpatrick et al. 2009).

Sexual conflict and NFDS

NFDS driven by temporary attentional bias is not limited to predator-prey interactions but can occur in a variety of antagonistic relationships (Gigord et al. 2001; Sherratt 2001). Because my focus is on sexually selected traits and particularly sexually selected colour patterns, I will expand on the role of sexual conflict and NFDS by visually driven males in maintaining discrete variation in female colour traits.

Sexual conflict arises whenever the fitness consequences of mating and reproduction differ between the sexes (Arnqvist and Rowe 2005). This is common across animals, as a consequence of the fundamental disparity in reproductive investment between the sexes. One sex (usually males) invests predominantly in fertilization opportunities and the other sex (usually females) invests largely in offspring provisioning and care (Bateman 1948; Trivers 1972; Parker 1979). As a result, males typically have shorter re-mating latencies than females and they compete more strongly against each other for access to females (Janicke et al. 2016). Under these circumstances, male reproductive success will sharply increase with their mating rate, whereas females would benefit less, if at all, from mating with multiple males (Fig. 3a).

Alleles that increase male fertilization rates at the expense of female lifetime reproductive success will be favoured in males, and foster the evolution of female alleles (at different loci) that aid them to avoid, resist or tolerate male reproductive efforts (Rice and Holland 1997). Sexual conflict over mating is therefore a particular case of inter-genomic contest evolution, in which male-beneficial alleles at one locus will increase the probability of mating as the outcome of an inter-sexual interaction, and female-beneficial alleles at another locus will decrease it.

This scenario could be reminiscent of the L-K process described earlier, with female resistance to mating having the statistical effects of a preference. Increased coerciveness of male mating attempts could become statistically associated with increased female resistance driving an endless evolutionary arms race between the sexes. However, an important difference is that female resistance is almost invariably under direct natural selection, for example, if it increases harm caused by pre-mating struggles (Mühlhäuser and Blanckenhorn 2002). Natural selection on female resistance cannot be overcome by an advantage of producing more persistent sons, because a decrease in resistance towards its naturally selected optimum will also reduce the optimal value of male persistence (Fig. 3b). Therefore, direct natural selection on female resistance will ultimately determine the equilibrium value of male and female traits (Gavrilets et al. 2001).

The conditions described above of course assume that adaptations in one sex can be matched by counteradaptations in the other sex. However, in some circumstances one of the sexes can get the upper hand in the conflict (Arnqvist and Rowe 2002). This is not to say that one sex 'wins' over the other, as the mean fitness of both sexes in a population are necessarily identical, when the primary sex ratio is equal to one (Fisher 1930). What I mean by an upper hand is simply that the effect on mating rate of a novel persistence or resistance allele is not offset by the evolution of a counter adaptation in the other sex.

Theory shows that females can achieve this by diversifying into discrete genetic clusters. Female diversification in relation to the traits that mediate pre-mating inter-sexual interactions might prevent males from specializing in mating with any female cluster (Gavrilets and Waxman 2002). This is because as males specialize in one of the female clusters, viability costs of mating for these females will cause an increase in the relative fitness of the alternative female type. As a consequence, sexual selection will favour males to switch their female targets. If demographic conditions preclude male diversification matching each of the alternative female clusters, then males will be caught in a sexual selection equivalent of the Buridan's Ass dilemma[5].

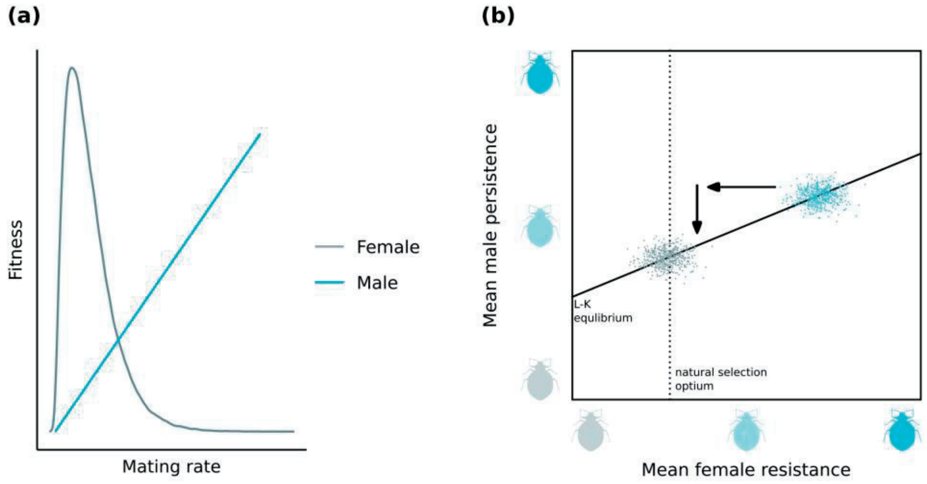

Figure 3. Sexual conflict over mating rate. **(a)** Sexual conflict over mating arises when the fitness of individual males increases with mating rate, whereas female fitness peaks at an intermediate mating rate. **(b)** In this scenario, loci underlying male persistence coevolve with the loci behind female resistance. However, unlike the L-K process of sexual selection by female choice, where equilibrium values occur along a line of matching male display traits and female preferences, direct natural selection on female resistance typically determines the point of equilibrium. Natural selection (horizontal arrow) will push a displaced population (bright blue) towards the naturally selected optima for female resistance (dotted line). The potential benefits to females of having very persistent sons will not overcome natural selection because by reducing their resistance females indirectly select for less persistent males (vertical arrow).

The Buridan's Ass regime means that males fail to evolve adaptations to secure matings with either female type. At any given point in time, males have the least incentive to target the rare female type, which will therefore be at an advantage. This does not necessarily mean, however, that individual males direct their mating efforts at random. If searching for females across a visually heterogeneous background is important to determine male mating success, then the formation of a temporary bias towards common female types would be advantageous for males, in a manner analogous to the predator-prey system described above. Under these

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[5] The Buridan's Ass dilemma is a philosophical paradox that illustrates the burden of free will. It depicts a donkey, which while being equally hungry and thirsty cannot decide between equidistant hay and water, and inevitably starves to death. In the sexual conflict scenario, males do not starve to death, but they are forced to remain equally adapted (and maladapted) to both female clusters. On an evolutionary time scale, males, like the donkey, fail to 'make a choice' between two nearly identical alternatives.

circumstances, females with rare colour patterns might be disproportionally overlooked by visually searching males, reducing their per capita sexual harassment and mating rates, and thereby increasing their lifetime reproductive success. Selection on female colour patterns would thus be negative frequency-dependent, because as the less pursued females increase in frequency their reproductive advantage is diminished.

Why good (and bad) things come in threes

So far, I have discussed mechanisms for the evolution and maintenance of discrete variation in colour traits with distinct roles in mediating inter-sexual interactions. An important empirical observation is that in many colour-polymorphic systems colour morphs are associated with a suite of behavioural, physiological and life-history traits (Mckinnon and Pierotti 2010; Llaurens et al. 2017). For example, red and black morphs of the Gouldian Finch (*Erythrura gouldiae*) differ in immune function, as well as in the degree of intra-sexual aggressive behaviour (Pryke et al. 2007). Three male morphs in the side-blotched lizard (*Uta stansburiana*) differ in their multivariate reproductive tactics (Sinervo and Lively 1996; Sinervo et al. 2000b). A dominant and aggressive orange-throat morph defends large territories with several females, has high stamina and low interannual survival. A sneaker yellow-throat morph has instead low stamina and steals copulations when unnoticed. Finally, a blue-throat morph with intermediate stamina guards a single female in a small territory. Similarly, territorial, satellite and female-mimicking reproductive tactics in the ruff (*Philomachus pugnax*) are associated with different colour morphs, which also differ in size (Lank et al. 1995).

Among females, the evolution of alternative life-history and reproductive tactics is probably under recorded (Neff and Svensson 2013), yet female colour morphs are also known to differ in mating tactics (Gosden and Svensson 2009), in their sensitivity to density-dependent interactions (Svensson et al. 2001), and in their immunological competence (Calsbeek et al. 2008). Thus, in both sexes, these phenotypic associations between multiple traits and discrete colour variation seem to be adaptive and in line with life history trade-offs. For example, aggressiveness and territoriality typically come together at a cost of higher immune sensitivity. The maintenance of discrete colour morphs within species is interesting in itself, but also because it can help us understand how and why these associations arise in the first place, and how easily can they be broken down or changed.

Correlational selection

The fitness effects of a trait often depend on its interactions with other traits. In garter snakes (*Thamnophis ordinoides*), frequent reversals of the direction of flight increase survival in striped individuals, as they create an optic illusion that hinder predator attacks (Brodie III 1992). The optical illusion effect requires both colour and behavioural components. Thus, in plain-coloured individuals, the same behaviour reduces survival. Selection that acts on such trait combinations is known as *correlational selection*, because the direction and strength of selection on one trait depends on the value of the other trait and *vice versa* (Lande and Arnold 1983). Unlike directional selection in which only one combination of both traits maximizes fitness, correlational selection can result in a ridge of high fitness defined by equally adaptive trait combinations, or alternative fitness peaks separated by a saddle of intermediate fitness (Fig. 4).

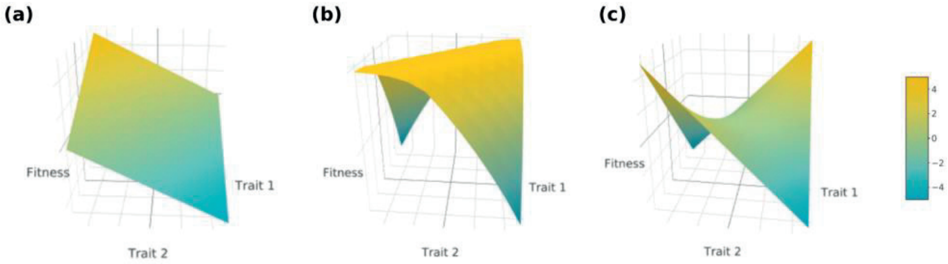

Figure 4. Hypothetical fitness surfaces under **(a)** directional selection acting independently on two traits, **(b)** correlational selection resulting in a fitness ridge of equally adapted trait combinations and **(c)** correlational selection resulting in two alternative fitness peaks. Note that if correlational selection favours negative correlations between traits, the lowest values of both traits are located in the front-right corner of the plots. Based on Agrawal et al. (2010).

Negative correlations between advantageous traits can arise for two reasons, both governed by correlational selection. First, two traits that would independently contribute to fitness may not be simultaneously maximized if they rely on a common resource or are limited by a shared developmental constraint. A classic example of this type of negative correlation is the life-history trade-off between egg size and number. While females would benefit from laying many large eggs, both traits strain a female's limited provisioning resources. The result of this trade-off is that females only maximize one of the traits either in response to the environment (Fox et al. 1997), or as part of a genetically determined life history strategy (Sinervo et al. 2000a).

Alternatively, correlational selection might favour combinations of traits that do not depend on a shared resource but are redundant in their fitness enhancing effects (Agrawal et al. 2010). Redundancy means that an increase in the expression of one trait causes relaxed selection on the other. Consequently, negative correlations between the traits can become adaptive.

The existence of distinct defence mechanisms against abiotic and biotic stressors can result in adaptive negative correlations. As an example, plants may evolve resistance mechanisms to prevent frost damage or tolerance adaptations that reduce the reproductive costs of having damaged tissues (Agrawal et al. 2004). However, neither trait is cost-free. An increase in resistance will act as selection against increased tolerance and *vice versa*, resulting in a negative correlation between the two. In the example of frost damage, intermediate values of both defence traits convey intermediate fitness. When both traits are expressed at intermediate values, an increase in either would causes a greater fitness difference compared to when one of the traits is nearly maximized. Therefore, correlational selection between these two fundamentally different types of defences results in a saddle-shaped fitness surface, where only combinations that maximize one trait at the expense of the other have high fitness (Agrawal et al. 2004).

Pleiotropy and epistasis

Correlational selection explains why trait correlations evolve (Sinervo and Svensson 2002), but not how the underlying genetic correlations that produce these co-adapted phenotypic variants are formed. In the case of discrete and heritable colour polymorphisms, a key question is how a suite of correlated traits can become linked to the loci that govern colour development. Two main non-exclusive explanations can account for this widespread phenomenon (reviewed in Mckinnon and Pierotti 2010).

The first type of explanation involves the formation of *supergenes*, which are DNA regions of low **recombination** that include two or more loci, each with effects on different traits. The combined effects of these loci increase the pleiotropy of the supergene as a whole. Supergenes can be formed by chromosomal rearrangements such as inversions, translocations and gene duplications (Schwander et al. 2014). For example, translocations and gene duplication (followed by functional divergence between gene copies) can cause the physical proximity of loci underlying co-selected traits. An inversion in the chromosomal region where these loci are located could also be favoured by correlational selection as it can prevent recombination of co-adapted alleles (Fig. 5a).

Alternatively, these trait associations can arise from the evolution of a limited number of regulatory loci, which influence the expression of the genes that code for traits under correlational selection (Fig. 5b). This has been referred to as *regulatory linkage*, because genetic correlations between co-adapted traits arise from the effect of a regulatory locus, even if the genes coding for these traits are not physically linked (West-Eberhard 2003). A key point about regulatory linkage is that unlike the supergene mechanism described above, the pleiotropic effects of the morph-determining locus are *necessarily* due to the effects of interacting genes, known as *epistasis*.

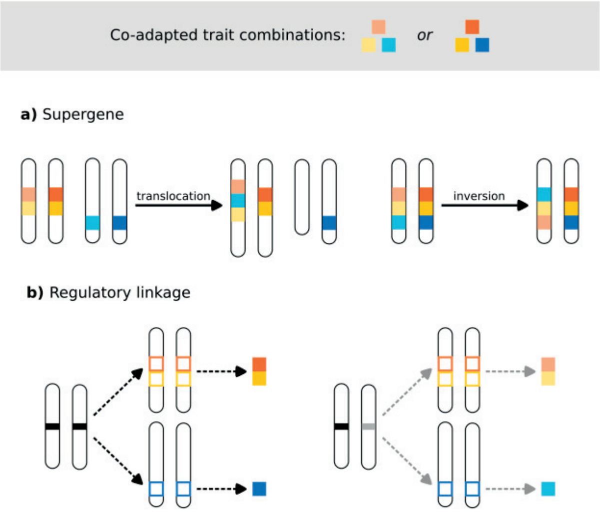

Figure 5. Schematic representation of alternative genetic mechanisms for the evolution of pronounced phenotypic differences between discrete morphs. **(a)** Morphs might differ in various phenotypic traits if the genes underlying these traits become locked in a DNA region of low recombination, forming a supergene. Chromosomal rearrangements, such as translocations and inversions, play an important role in the formation of these supergenes. **(b)** Multivariate phenotypic differentiation can also be caused by allelic variation in a top-regulatory locus linking alternative expression patterns across multiple genes. In this case, epistasis plays a fundamental role in the distribution of phenotypic variation among individuals. Because regulatory genes can be affected by chromosomal rearrangements, these alternatives are not mutually exclusive.

Epistasis has different meanings for different kinds of biologists (Wade 1994). For molecular biologists, epistasis occurs whenever the function of a gene product depends on another previously or simultaneously expressed locus. The function of an enzyme which depends on the presence of a cofactor is an example of *physiological epistasis*, because the enzyme-coding genes are different from the genes that control the synthesis of the cofactor. In population genetics, *statistical epistasis* occurs if the phenotypic effects of a gene depend on which alleles are present at other loci in the genome. Thus, unlike physiological epistasis, statistical epistasis requires the existence of genetic variation in at least one locus.

The evolution of pronounced and multifarious phenotypic differentiation between heritable morphs via regulatory linkage illustrates both concepts of epistasis. Regulatory genes are by definition involved directly or indirectly in physiological epistasis with multiple other loci, which in this case control the expression of a number of co-selected traits. Allelic variation in this top-regulatory morph locus determines variation in colour and in other co-selected traits, thereby producing statistical epistasis. Regulatory linkage is a very appealing mechanism for explaining the presence of complex suites of traits associated with heritable morphs, because it does not require multiple chromosomal mutations bringing together previously unlinked genes (Charlesworth and Charlesworth 1975). However, it begs the question of how does pervasive epistasis evolve. In other words, how can allelic variants at a single locus have widespread regulatory effects on multiple other loci across the genome?

The answer probably lies on the feedback between genes and hormonal systems. Hormones respond to and mediate gene expression. Genes that influence the sensitivity to hormones at specific times or in specific tissues can have profound effects on the development of alternative phenotypes (McGlothlin and Ketterson 2008). For example, in the side-blotched lizard mentioned at the beginning of this section, a single morph locus interacts with the hypothalamic-pituitary-gonadal hormonal axis, which in turn influences multiple behavioural, physiological and life history traits. Such traits include mate acquisition behaviour and stamina in males, fecundity and offspring quality in females and immune function in both sexes (Sinervo and Calsbeek 2003; Svensson et al. 2009).

The feedbacks between hormones and genes are clearly complex, yet they underscore the value of colour-polymorphic animals to study how these regulatory mechanisms evolve in response to correlational selection. This is because colour polymorphisms often provide visually identifiable markers for suites of multifarious traits that are maintained by correlational selection. By documenting trait correlations among these morphs and examining how a few colour-morph loci interact with the genes underlying these traits, we can gain valuable insights on the role of epistasis in adaptation and in the evolution of phenotypic variation.

Colour variation across life stages

The effects of genes depend on the environment. In the previous two sections I have shown this for external social environments, i.e. how many individuals of each phenotype are present, and for internal environments determined by gene products from other loci in the same genome. Another fundamentally important dimension of environmental variation that influences the fitness consequences of alternative phenotypes is variation that occurs throughout the lifetime of an individual. Often, organisms can develop alternative phenotypes from their unique genotype in response to environmental changes. This ability is termed *phenotypic* *plasticity* (West-Eberhard 1989). If the plastically induced phenotypes match the selective regimes in place, then such plasticity is adaptive.

The flexible and adaptive expression of colour traits in response to environmental cues has been studied for several decades, particularly with respect to external ecological environments. For example, wing eyespots are developed and disappear in response to temperature in a butterfly (*Bicyclus anynana*) that is viewed by its predators against different visual backgrounds between wet and dry seasons (Brakefield et al. 1996; Lyytinen et al. 2004). The visual environments where these butterflies live as adults vary markedly between seasons, resulting in an advantage for a plain brown form in the dry season and an advantage for a deflective colour pattern, including wing eyespots, during the wet season (Lyytinen et al. 2004). These alternative forms are induced in response to temperature, which also varies between seasons, and during a critical phase of late larval development (Kooi and Brakefield 1999). Therefore, plasticity in the development of wing colour patterns is adaptive, as specific phenotypes that match the forthcoming selective regime are developed in response to environmental cues.

Phenotypic plasticity in the expression of sexually-selected colour traits, apart from the condition-dependent expression of male display traits, has received considerably less attention. The few examples include plasticity in the expression of sexually-selected pigment-based colour traits in guppies in response to predator cues, given that predation risk is higher for pigmented males (Ruell et al. 2013), and male-specific colour changes during explosive breeding in moor frogs (*Rana arvalis*), presumably as such changes facilitate mate recognition during scramble competition for mating opportunities (Sztatecsny et al. 2012); Fig. 6a). Perhaps this disparity of evidence indicates that sexually selected colour traits tend to be more developmentally stable (i.e. more canalized) than naturally selected colour patterns. But it could also reflect a long-standing research tradition focusing on signals expressed in males in competition for access to females (Edward and Chapman 2011).

Developmental plasticity in the expression of colour traits may be more important for females than for males, because females might often be more sensitive to the timing of inter-sexual interactions. As mentioned above, females typically make a greater investment in offspring provisioning and care than males do (Trivers 1972; Janicke et al. 2016). Thus, females are often unreceptive for longer periods of time than males, in order to acquire resources and provide care. This has been traditionally recognized to result in a male-biased sex ratio at places where mating occurs (Clutton-Brock and Vincent 1991). However, it can also result in a more temporally dynamic sexual selection regime for females, as the costs and benefits of mating will change with their own internal physiological environment.

Females of many animal species express developmentally plastic colour signals in association with the onset of reproductive capacity, ovulation and changes in mating status (Rowland et al. 1991; Weiss 2002; Setchell et al. 2006; Barelli et al. 2007; Tannure-Nascimento et al. 2008; Takahashi and Watanabe 2011; Fig. 6) However we know little about why and how variation in these signals arises. Because males presumably also benefit from avoiding reproductively unsuitable females, there is no expectation that this type of female colour signals will become increasingly or differentially elaborated, in the way that male signals used in female choice do (see section *Classic explanations for colourful traits*).

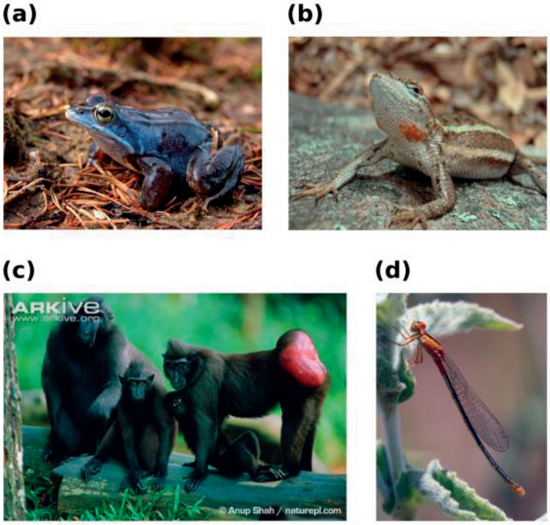

Figure 6. Examples of sex-limited colour traits with developmentally plastic expression. **(a)** Male moor frogs (*Rana arvalis*) develop a blue colouration during the mating season (Sztatecsny et al. 2012). **(b)** Red colour patches in females of the striped plateau lizard (*Sceloporus virgatus*) are expressed in association with sexual receptivity (Weiss 2002). **(c)** Females of many primate species display conspicuous sexual swellings in association with ovulation (Nunn 1999), here shown in the Celebes crested macaque (*Macaca nigra*). **(d)** Females in many species of forktail damselflies (genus *Ischnura*) undergo dramatic colour changes during adult development. In *Ischnura senegalensis*, these colour changes are thought to be used by males to identify suitable partners. Here, the immature colour phase of a related species (*Ischnura intermedia*) is illustrated. The final female colour phase in both species is dark green and black. Image credits **(a-d)**: Hans Dekker (Saxifraga Foundation), Sarah Zielinski (www.smithsonianmag.com), ©Anup Shah (naturepl.com), Rose Sparrow.

Sexual conflict over mating and parental care might promote variation in female sexual colour traits, including the evolution of deceptive signals. Theory predicts that a greater risk of infanticide in primate groups should result in greater intensity of female sexual signalling, as a means to bias paternity towards the type of males that would otherwise pose such a threat (Rooker and Gavrilets 2018). For many animals, mating is a costly activity as it increases the risk of predation (Zuk and Kolluru 1998), sexually transmitted disease (Thrall et al. 1997) and may even be physically harmful (Chapman et al. 1995; Stutt and Siva-Jothy 2001). Thus, variation in the developmentally plastic expression of female signals could also be explained by variation in the intensity of sexual conflict over mating rates, with females evolving signals to advertise their reproductive unavailability in contexts of high male pre-mating harassment.

To the best of my knowledge, the latter possibility has not been empirically or theoretically addressed, but it is not inconceivable, given that empirical evidence exists for the role of developmental signals in avoiding male-male antagonistic interactions. In some bird species, for example, resource-limited males are thought to adaptively delay their plumage colour maturation, in order to reduce aggression from more dominant males (Hawkins et al. 2012). I suspect that the importance of developmental plasticity in modulating inter-sexual interactions and sexual conflict can be further understood by investigating the relation between key developmental events and sexual signalling in females.

The tug of war between natural and sexual selection

Colour traits often act as communication signals that mediate pre-reproductive interactions, but also influence the risk of detection and recognition by potential enemies such as predators and parasites (Zuk and Kolluru 1998). As noted at the beginning of this introduction, male colour patterns (i.e. display traits) may indicate nothing to females other than their availability to mate. Yet, because there is a limited number of ways in which the background can be matched, even a L-K mechanism of arbitrary trait-preference coevolution will often result in colouration patterns that are conspicuous against the background for both conspecifics and predators.

In Trinidadian guppies, males from streams with and without predators vary dramatically in the expression of sexually-selected bright colouration (Endler 1978), and females have a stronger preference for conspicuous colour patterns in predator-free environments (Breden and Stoner 1987). Within populations, theory predicts that environmental heterogeneity and microhabitat segregation of natural and sexual selection can create conditions in which male-limited colour polymorphisms can be maintained (Chunco et al. 2007). In empirical studies, the opposing effects of predators and female mate choice have been speculated to play a role in the maintenance of male-limited colour polymorphisms in other species of fish, in which mating preferences and predation risk (Kingston et al. 2003), or visual environments (Gray et al. 2008) differ among habitats. Furthermore, in aposematic wood tiger moths (*Arctia plantaginis*) two male colour morphs, which respectively benefit from survival and mate choice advantages, are presumably maintained in part due to a trade-off between mating success and survival that is spatially variable (Nokelainen et al. 2012; Nokelainen et al. 2014).

These studies show that conflicting selective forces that act on colour traits and vary spatially may influence the evolution of sex-limited morphs. I think that the importance of pre-reproductive selection in shaping the evolution of discrete colour morphs could be further understood by looking beyond selection on colour traits *per se*. Since the genes governing colour-morph development typically have pleiotropic effects (see previous sections), ecologically relevant traits that are genetically correlated with colour and are expressed earlier in ontogeny may influence the proportions of the different morphs among reproductive individuals. In other words, even if the influence of colour on mate choice is independent from ecology (*sensu* Kirkpatrick 1987), colours that are selected by potential mates could be opposed by natural selection if the development of such colours compromises traits such as immunity, thermoregulation or secondary defences in certain environments. Thus, the theoretical predictions of Chunco et al. (2007) might also be of biological relevance in cases where traits co-regulated with colour development (see section *Pleiotropy and Epistasis*) provide differential viability across environments.

The situation modelled by Chunco et al. (2007) required unrestricted movement between habitats with distinct natural and sexual selection regimes. This assumption may be unrealistic for many animals with limited dispersal and which occupy patchy environments. It is therefore probable that most lasting polymorphisms are maintained locally, and by frequency-dependent mechanisms, which commonly operate among reproductive individuals (e.g. Sinervo and Lively 1996; Gigord et al. 2001; Olendorf et al. 2006; Takahashi et al. 2010; Bots et al. 2015; Le Rouzic et al. 2015). This does not mean, however, that pre-reproductive selection on correlated traits would be unimportant in those species. Instead, environmentally heterogeneous pre-reproductive natural selection could drive population differences in morph composition and potentially influence geographic range differences between monomorphic and polymorphic **taxa** (Forsman et al. 2008).

Recording patterns of opposed selective pressures at different life stages in the field is key to understanding the evolution of life history trade-offs (Schluter et al. 1991). To this end, heritable colour morphs might be particularly useful as visual markers of suites of genetically correlated traits underlying these trade-offs. Investigating how environmental heterogeneity influences natural selection on sets of traits correlated with discrete colour morphs can help us understand the mechanisms that maintain phenotypic variation within and among populations.

A phylogenetic digression

Natural and sexual selection work with what they have. They operate in populations that have been influenced by past selective regimes and demographic processes. When evolutionary biologists seek to investigate macroevolutionary patterns of trait distributions they must ask to what extent similarities between species reflect shared historical contingencies as opposed to shared selective pressures. **Phylogenies** provide information about the shared history of lineages which are evolving independently at present. Therefore, robust phylogenetic inference is a cornerstone not just of systematics but to all modern evolutionary biology.

Phylogenies are also important because they allow us to describe the temporal and spatial dynamics of diversification trajectories for different branches of the tree of life. Such information is crucial for studying macroevolutionary change in phenotypic traits. This can be illustrated by studies of potential adaptive radiations. Adaptive radiations occur when organism diversify rapidly, filling up a multitude of ecological niches. Thus, tests of the predictions of adaptive radiation require quantifying the rates of both phylogenetic diversification and morphological evolution (Burbrink et al. 2012; Hopkins and Smith 2015).

The recent explosion of technological advances for molecular sequencing has stimulated a wave of methodological developments to cope with this wealth of data (e.g. Höhna et al. 2016; Wen et al. 2016). However, some of the challenges of inferring past diversification events may not be overcome by simply increasing sequencing volume. These challenges include inferring the timing of speciation events in an absolute timescale. When phylogenetic trees are inferred from molecular data, evolutionary divergence between taxa is expressed in units of molecular substitutions accumulated since their most recent common ancestor (Fig. 7a). The molecular clock hypothesis posits that the amount of molecular divergence between taxa will be equal to the product of the clock-like rate of molecular evolution[6] and the time since their phylogenetic divergence (Zuckerkandl and Pauling 1962). The assumption that all lineages share a single (strict) clock rate may be relaxed for more distantly related taxa, hence allowing different rates in different branches of the tree (Fig. 7; Drummond et al. 2006). However, in either case, speciation times are only relative measures of divergence

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[6] The molecular clock hypothesis can be rationalized under the neutral theory of molecular evolution, which posits that the rate of molecular evolution will be equal to the neutral mutation rate, due to the random fixation of neutral or nearly neutral mutations (Kimura 1968). If the mutation rate is equal among lineages, a constant evolutionary rate is expected, and therefore, sequence divergence between taxa will be roughly proportional to divergence time.

time between taxa, unless external information is provided for the absolute timescale of the tree.

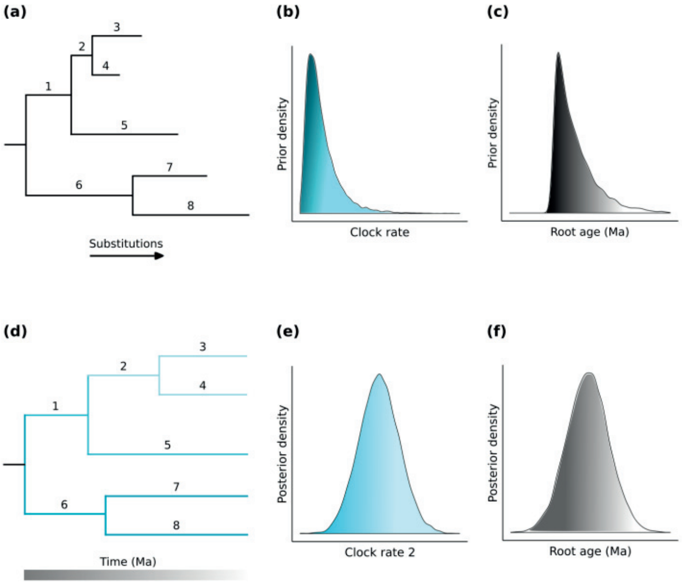

Figure 7. Schematic representation of phylogenetic dating under a relaxed clock model and an empirical root prior. **(a)** Without extrinsic information, branch lengths in a phylogeny are proportional to the inferred number of molecular substitutions. Under a relaxed clock model, clock rates for each branch (1-8) are drawn from a prior distribution. In this case all branches are drawn independently from a log-normal **(b)**. One or more empirical priors are typically used to calibrate specific nodes in an absolute time scale. In this example, the prior density on the age of the root follows an exponential distribution with an offset (hard minimum) determined by the age of a fossil **(c)**. The branching process (i.e. tree prior), which is scaled by the root age, and the clock model for the branch rates are combined in a Bayesian framework. Numerical methods, such as Markov-Chain Monte Carlo are used to approximate the posterior probability distribution of the model parameters, given the molecular sequence data (see Methods). These parameters determine **(d)** the branch lengths now proportional to absolute time, and include **(e)** the clock rates, here shown for branch 2, and **(f)** the root age.

Traditionally, this external information comes from a calibration density, based on the age of a fossil with known phylogenetic affinity or a paleogeographic event, such as the formation of an island or a dispersal barrier (Fig. 7c). While these approaches have granted substantial advances in elucidating the evolutionary history of many groups of plants and animals (e.g. Moreau et al. 2006; Bininda-Emonds et al. 2007; Smith et al. 2010) they also suffer from shortcomings that might be particularly severe for some clades. For example, many organisms do not fossilize well. For those clades that do have an adequate fossil record, model comparison is complicated by the fact that the shape of prior densities on divergence times are typically defined by virtue of an expert's opinion, which often lack justification (Warnock et al. 2011). The same complication regarding prior densities permeates biogeographic calibrations, which also suffer from the problem of 'double counting the data' (Landis 2017). The latter situation occurs when the distribution of extant taxa, for instance on different islands, is used once to justify the node calibration and again for the biogeographic inference.

Recently, these problems have been addressed in a series of efforts to transition from phenomenological to mechanistic approaches for phylogenetic dating. The methods for tip dating and the fossilized birth-death process have allowed the inclusion of fossil information as part of an evolutionary generative process, as opposed to an empirical prior. Tip dating approaches incorporate fossils as terminal taxa, and model the evolution of morphological characters, measured in fossil and extant taxa, in a standard phylogenetic framework (Pyron 2011; Ronquist et al. 2012). The fossilized birth-death process incorporates fossil information in a single diversification model in which the posterior rates of lineage speciation, extinction and fossilization are induced by all available data on extant species and known fossils (Heath et al. 2014).

Landis (2017) recognized that the essence of the generative process introduced by Heath et al. (2014) can be used to incorporate other sources of dating information, as long as there is an underlying stochastic process that is jointly applied to the calibration and molecular data. Thus a model that jointly considers alternative biogeographic and diversification scenarios can be applied to a variety of taxa as an alternative or a complement to fossil data. This approach opens up opportunities for dating phylogenies of groups of organisms that do not have a rich fossil record in a robust statistical framework. It also makes it feasible to address phylogeographic questions while accounting for phylogenetic uncertainty. I used this approach, further detailed below, to infer a phylogenetic hypothesis for pond damselflies, and hence to be able to address questions regarding the temporal and spatial patterns of diversification and dispersal across the history of the entire clade.

On the origin of morphs

An important goal in evolutionary biology is to investigate to what extent microevolutionary processes explain phylogenetic patterns of phenotypic distributions, and therefore to link ecological and evolutionary time scales (Weber and Agrawal 2012). With the advent of molecular phylogenies for an increasing variety of taxa, and a recent statistical and computational developments for phylogenetic comparative analyses (reviewed in Garamszegi 2014), the opportunities to address this question are literally at our fingertips. In the last two decades, evolutionary biologists have made use of such growing arsenal of analytical tools to tackle this issue in two main ways: first by investigating the incidence of functional or morphological convergence, and second by assessing correlations between putative selective pressures and phenotypic variation at a macroevolutionary scale.

Evolutionary convergence is often thought of as evidence of the repeatability of evolution by natural selection, if similar morphological or ecological features do arise as a consequence of similar selective environments in different locations and different clades (Losos 2011). Traditionally, studies of convergence have focused on young and spatially segregated radiation such as the Caribbean anoles (Losos et al. 1998; Mahler et al. 2013) and East Africa cichlids (Muschick et al. 2012). However, recent studies have shown that evolutionary convergence may even be rampant in old and globally distributed clades, yet imprinted by history. Moen et al. (2015) showed that in frogs, a clade that is over 150 million years old, morphological convergence in different communities across the planet is driven by similarities in microhabitat use, which has changed frequently throughout their evolutionary history.

The second approach for linking microevolutionary processes and phylogenetic patterns looks at whether phenotypic variation correlates with environmental variation at a macroevolutionary scale. For example Mesquita et al. (2016) found that clutch size and reproductive frequency in lizards are correlated with temperature and precipitation variation at a global scale. Their results suggested that frequent reproduction with small clutches is advantageous in tropical and aseasonal environments, whereas viviparity is more likely to evolve in harsher environments of temperate areas and higher latitudes.

These studies show that comparative analyses can help elucidate the relative contributions of shared ancestry and shared selective pressures, over broad phylogenetic scales. Such analyses, of course, require a measure of the relevant selective environments, which in many cases, as the example above, correspond to abiotic factors. This is probably due to the practical reason that data on ecological variables, such as temperature or precipitation, can be obtained for a large number of species from global databases, given some information on distribution ranges. Sexual selection can also be a powerful engine of phenotypic evolution, but given that sexual selection is a social process, demographic variables often provide better measures of sexual selection regimes. Quantifying variation along these axes for a large number of taxa is a daunting task, in contrast to quantifying the abiotic factors mentioned above. Consequently, most studies addressing the importance of social selection in shaping phenotypic variation at a macroevolutionary scale are based on the most well-known taxa, that is birds and mammals (e.g. Liker et al. 2013; Lukas and Clutton-Brock 2013), or use abiotic factors as proxies of sexual selection, when links between the two are reasonably well established (e.g. Machado et al. 2016).

In addition to this limitation, phylogenetic comparative studies are rarely designed to address specific predictions arising from experimental and observational studies at the scale of populations and species (e.g. McGlothlin et al. 2018), although such an approach has been advocated as a fruitful avenue for integrating micro- and macroevolutionary perspectives (Weber and Agrawal 2012). The evolution of discrete phenotypic variation, such as colour morphs, provides a suitable opportunity to attempt such integrative approach. As noted previously, the social selective environments that maintain such form of phenotypic variation are relatively well understood (e.g. Sinervo and Lively 1996; Olendorf et al. 2006; Le Rouzic et al. 2015). Therefore, these polymorphic organisms, if common, can be used to investigate if the selective environments that maintain phenotypic variation within populations can also explain the origin of novel phenotypic variants and if there are common themes in the phenotypes of these novel morphs.

In the case of female-limited morphs, which may evolve due to sexual conflict over mating (Gavrilets and Waxman 2002), phylogenetic comparative analyses can help clarify the role of social selection as a driver of the macroevolution of phenotypic diversity. Social selection encompasses all instances of traits favoured due to their effects on other individuals (West-Eberhard 1983). Social selection is therefore density- and frequency-dependent, because these effects are only relevant in relation to the effects of alternative phenotypes. As I discussed at the beginning of this introduction, the role of positive frequency-dependent selection in the evolution of male signalling traits has been increasingly recognized, as a mechanism that can result in both trait exaggeration and fast diversification (Prum 2017). Colour traits that help females avoid male harassment are also under frequency-dependent selection, but the effects of increasing frequency are typically negative. Yet, both mechanisms, positive frequency-dependent selection in male signals and negative frequency-dependent selection on female anti-harassment traits share the relentlessness of social regimes, where no phenotype may be good for long. While this similarity suggests that social selection on females may also be a potent driver of diversification, the macroevolutionary consequences of sexual conflict on female-limited variation remain largely unexplored in a phylogenetic framework.

Aims of the thesis

In this thesis I use colour-polymorphic pond damselflies to address the following questions:

1. How is phenotypic variation in defence traits and developmental trajectories distributed among discrete colour morphs? **Paper I, II and III**
2. Do morphs differ in the relative timing of crucial developmental events? **Paper II**
3. What is the role of morph-specific developmental colour changes in pre-mating interactions? **Paper III**
4. How do developmental sexual signals evolve in females? **Paper II and III**
5. What is the role of pleiotropy and pre-reproductive natural selection in shaping spatial variation in morph frequencies? **Paper IV**
6. When and where did pond damselflies evolve and how did they acquire their current patterns of global species richness? **Paper V**
7. Is the macroevolutionary origin of female-limited colour polymorphisms associated with sexual conflict over mating rates? **Paper VI**
8. What are the macroevolutionary consequences of female-limited colour polymorphisms? **Paper VI**

General Methods

Study systems

I studied different aspects of sex-limited colour variation in damselflies (order Odonata, suborder Zygoptera). In **Papers I – IV**, I focused on a single well-known species, the Common Bluetail (*Ischnura elegans*). Females of *I. elegans* occur in three discrete and heritable colour morphs, whereas males are monomorphic (Fig. 8a). This polymorphism is governed by a dominance hierarchy at a single locus or a set of tightly linked loci, with Mendelian segregation and sex-limited expression (Cordero 1990). Therefore, the three alleles, often denoted as *p*, *q*, and *r* produce six possible genotypes and three alternative morphs (Svensson et al. 2009).

In *I. elegans*, females with genotypes including the dominant allele (*pp*, *pq* and *pr*) develop into a morph with a male-like colour pattern at maturity. These *Androchrome* females (hereafter A-females) are often referred to as male mimics, as their male-like appearance (Fig. 8), in this and other species with a similar polymorphism (Robertson 1985; Gosden and Svensson 2009), is thought to result in reduced male harassment in comparison to other female morphs which are markedly different from males (i.e. sexually dimorphic). Two other genotypes (*qq* and *qr*) result in a female morph which is sexually dimorphic at maturity and is traditionally known as *Infuscans* (hereafter I-females, Fig. 8). The final genotype (*rr*) develops into *Insfuscans-obsoleta* (hereafter O-females), an alternative morph that is also sexually dimorphic at maturity (Fig. 8).

Previous population and behavioural studies indicate a predominant role of NFDS via male mating and pre-mating harassment for the maintenance of the female polymorphism within populations. Male mating preferences change with female-morph frequencies (Van Gossum et al. 2001), despite a baseline preference for I- over A- females, as the latter ones are inherently less fecund (Gosden and Svensson 2009). Excessive male harassment reduces female fecundity, as shown by an experimental mesocosm study in which overrepresentation of either of two female morphs resulted in a decrease in overall female fecundity (Takahashi et al. 2014). In line with this scenario of sexual conflict over mating and flexible male search-image formation, morph frequencies exhibit a signature of stabilizing frequency-dependent selection over long time series and across multiple populations (Le Rouzic et al. 2015). Taken together, these studies strongly support the idea that NFDS via male sexual harassment is the main mechanism preventing the local loss of morphs by genetic drift or pre-reproductive selection. This idea is further supported by the fact that across surveys of 90 European populations of this species, none is monomorphic and a few are dimorphic (Gosden et al. 2011).

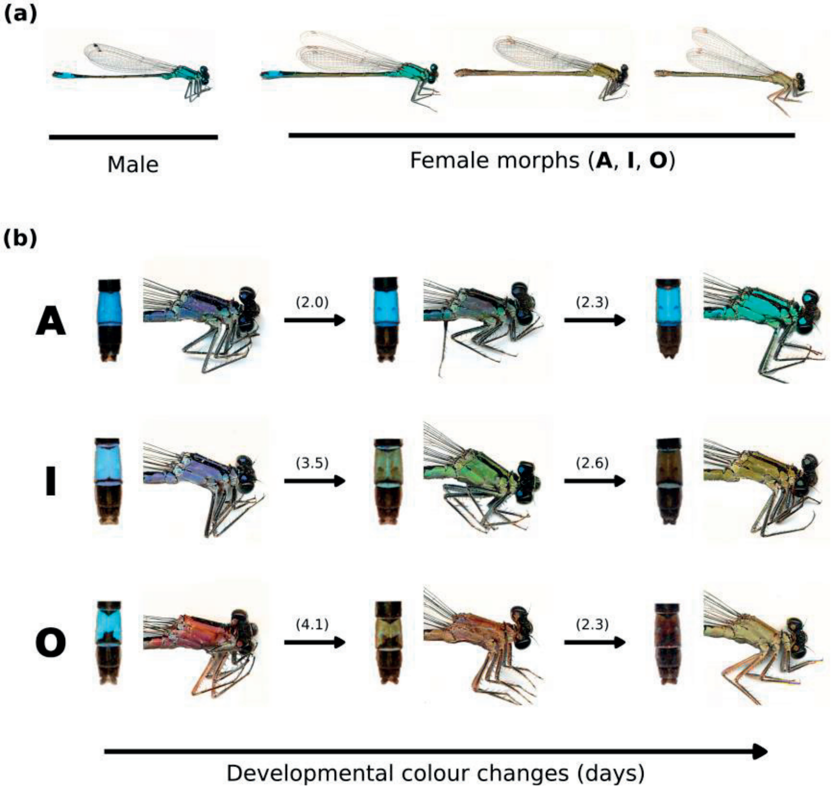

Figure 8. Heritable morphs **(a)** and female-limited colour changes **(b)** in *Ischnura elegans*. Colour changes are depicted for the thorax and distal abdominal segments in each morph. The mean number of days between developmental colour phases is shown in parenthesis.

Casual observers of insects probably think of damselflies as small but rather elongated fliers, typically found in or near freshwater habitats such as ponds and streams. Most damselflies lay their eggs in aquatic vegetation or in floating detritus and hence larvae develop within these aquatic environments. Larvae emerge onto land upon their last moult, from which the winged adults eclose. Adults will not moult again, but this does not mean they have stopped developing. Adult females are not sexually mature upon emergence and a few days will pass before the cells in their ovaries (oocytes) form mature eggs. In many species of damselflies, including *I. elegans*, females undergo sex and even morph-specific colour changes during this period (Fig. 8b).

In *I. elegans*, all sexually immature female morphs share a distinct blue colour patch in the distal portion of their abdomen, which is also present in males (Fig. 8). Over the course of sexual development, morphs undergo noticeable colour changes, in particular, extensive melanisation of coloured areas in the two sexually dimorphic female morphs (I- and O-females; Fig. 8b). As a result, the blue patch becomes darkened and no longer observable in I- and O-females, but it is retained in the male-like A-females. This stark variation in developmental colour changes, in which only some females remove a potential immature signal over the course of sexual development, is not unique to *I. elegans*. The genus *Ischnura* comprises about 75 species. Of the 41 species I studied, several (~ 60%) are female-polymorphic, and in most (~ 80%), females of at least one morph are known to undergo dramatic colour changes throughout development. However, it remains unclear, how often do immature colour patterns include male-like elements, and why these colour traits evolve in otherwise sexually dimorphic females.

The genus *Ischnura* is part of the most speciose clade of damselflies worldwide, the superfamily Coenagrionoidea. A recent molecular systematics study indicated that Coenagrionoidea is composed of two clades currently treated as families by taxonomists: Coenagrionidae, the pond damselflies and Platycnemididae, the featherlegs (Dijkstra et al. 2014). There are approximately 1 805 species in Coenagrionoidea, ~ 75% of which are pond damselflies. This superfamily is also characterised by remarkable interspecific colour variation (Fig. 1), in addition to the intraspecific heritable morphs and developmental colour phases described above. Early studies have shown that female-limited colour polymorphisms are widespread in this clade, at least among subclades with temperate distribution (Fincke et al. 2005). In contrast, to the best of my knowledge, no case of heritable male-limited colour polymorphisms or polymorphisms shared between the sexes have been reported in this superfamily.

These anecdotal observations suggest that pond damselflies and their relatives the featherlegs could provide exciting opportunities to explore the macroevolutionary patterns in the distribution of female-limited polymorphisms. However, until now there has been no phylogenetic framework to address these questions at a global scale. A global phylogeny is important because the only studies to date attempting to elucidate phylogenetic patterns in the occurrence of female morphs have focused on temperate lineages (Fincke et al. 2005; Van Gossum and Mattern 2008), although as in many other groups of organisms, most pond damselflies and featherlegs live in the tropics.

Surveying natural populations

A common damselfly

Some of my work (**Papers I - IV**) used data from an ongoing long-term population study of *I. elegans* in Southern Sweden. This study was initiated almost two decades ago by E.I.S., and has since recorded variation in morph frequencies within and between populations (Fig. 9). Every year since 2000, *I. elegans* populations are visited regularly during the mating season (June - August). In these visits, individuals are collected as they are encountered and the sampling effort (in minutes) is logged. Collected individuals are then brought to an indoor lab where their phenotypes are recorded. These phenotypes include the sex and thoracic colour of every individual and for females, the discrete colour morph and developmental colour phase. The database includes information on whether individuals were caught while mating since 2000. Since 2001, with the exceptions of a few years, females were kept individually in order to quantify their reproductive success. Today, this unique data set includes records from 18 natural populations, each surveyed during 2 – 18 seasons, and a grand total of over 50 000 phenotyped individuals.

Figure 9. My first field season (2014) in Southern Sweden with E.I.S, who initiated this longitudinal population study in 2000 and has maintained it since. Photo: Lukazs Bielawski

Many rare damselflies

Three studies in this thesis (**Papers III**, **V** and **VI**) built on a macroevolutionary perspective. I obtained hundreds of specimens from museums and private collections from enthusiastic odonatologists around the world (see Acknowledgements). I also reviewed the literature to gather phenotypic, ecological and demographic information on as many species of Coenagrionoidea as possible. Despite these efforts, it was clear that taxa in the temperate areas of the globe were overrepresented in both my phylogenetic and phenotypic-ecological datasets. Thus, in order to infer the phylogeny of pond damselflies and their relatives, and to address questions about the macroevolution of female-limited colour traits, we surveyed multiple populations in a standardized way and in four different continents[7]. Damselfly populations in Sweden, Guyana, USA, Argentina, Cyprus and Cameroon were surveyed for one or more of these three studies (Fig. 10). These surveys followed the same general methods as in the *I. elegans* studies mentioned above, except that female fecundity was not recorded and we collected specimens of all species occurring in every aquatic microhabitat (e.g. ponds, streams, marshes, forest swamps) that we visited.

Figure 10. Collecting and phenotyping pond damselflies near Mbam et Djerem, Cameroon, with the amazing Hanna Bensch and Yassin Tchinda. Photo: E.I.S.

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[7] A total of 107 populations in 6 countries were surveyed for **Paper VI**. Robin Pranter conducted all surveys in USA (10 populations). I led the remaining field work with the help of E.I.S, M.C.D. and several others (see Acknowledgements).

Experimental manipulation of developmental signals

I used phenotypic manipulations and behavioural trials to investigate the effects of female-limited colour changes on inter-sexual interactions in *I. elegans* (**Paper III**). Specifically, I asked if the blue abdomen patch that is present in immature females of all morphs, but is lost over development in the two most sexually dimorphic female morphs, could play a role in mediating pre-mating male harassment. If this male-like colour trait discourages male mating attempts, sexually immature females of all morphs could benefit of displaying such a signal. After confirming that immature-coloured females indeed have much reduced fecundity, we manipulated the apparent reproductive status of immature and matured-coloured females, by painting on their abdomens and subsequently observing male preferences. For each female morph and developmental colour phase, we conducted trials in which males had a choice between two females, one seemingly mature and one seemingly immature, and asked if males biased their attention towards mature-looking females, regardless of the female morph and actual age.

Developmental transcriptomics

I used transcriptomics as a tool to study morph-specific developmental trajectories over the course of colour changes (**Paper II**). For genes to influence the development of a phenotype they first need to be transcribed into mRNA. A transcriptome is the collection of all genes thus transcribed at a given time. We sampled females of each morph at three distinguishable points during colour development and sequenced their transcriptomes to ask how colour development aligns with other developmental processes. This approach allowed us to investigate which developmental changes are shared among female morphs during this window, and which are differentially regulated as a consequence of epistatic interactions between the colour morph locus and other loci.

In a nutshell, a differential gene expression (DGE) analysis has three steps: quality control, mapping and statistical modelling of transcript counts. Quality control is made to ensure that only reliable sequence data is used in the analyses and to determine if any sequencing artefacts have been introduced. The curated reads are then mapped to a reference genome or transcriptome, in order to count the number of transcripts for each gene isoform (i.e. each alternatively transcribed version of a gene). The number of transcripts associated to a single gene that are present in a cell or organism at a given time is therefore a direct measure of how much the gene is being ‘used’. There was no available reference genome for *I. elegans*, so the reference transcriptome was assembled from a collection of transcriptomes sequenced for this and other studies, using commonplace bioinformatic tools. Finally, transcript abundances are modelled statistically, in this case to determine if gene expression levels varied between colour-development stages, and if developmental regulation of gene expression differed between morphs. By comparing putative genes in the *I. elegans* reference transcriptome to genes with established molecular functions in other organisms, we can start to understand the functional consequences of developmental regulation of gene expression and its variation among heritable female morphs.

Bayesian model inference

Most results in this thesis are derived by Bayesian inference. Here, I will brush over the features of the Bayesian method and its implementation by Markov-Chain Monte Carlo (MCMC), in order to highlight the reasons why this approach is particularly suited for the questions I aimed to address. The main feature of Bayesian statistical inference is that all unknowns, including the model parameters, are treated as random variables, generated by some underlying probability distribution. This probability distribution reflects our uncertainty about the parameter values. The goal of a Bayesian analysis is therefore to let the data narrow down our initial uncertainty about the parameters of interest.

Let X be the observed data and θ an unknown model parameter. The Bayes theorem takes the form:

$$f(\theta|X) = \frac{f(\theta)f(X|\theta)}{f(X)} = \frac{f(\theta)f(X|\theta)}{\int f(\theta)f(X|\theta)d\theta} \qquad (1)$$

We are of course interested in $f(\theta|X)$, the probability density of parameter values after observing the data. This is the *posterior distribution*. The Bayes theorem shows that the *posterior distribution* is proportional to the product of the *prior distribution* of parameter values $f(\theta)$, which reflects our initial uncertainty, and the conditional probability of the data given the model $f(X|\theta)$, which is equivalent to the *likelihood* function. The marginal likelihood of the data, $f(X)$ is the probability of observing the data independently of the model. It is a normalizing constant to make the posterior probability integrate to 1, and is itself a high-dimensional integral, completely intractable for any model of meaningful biological complexity.

How do we then approximate the posterior distribution? We can do so by sampling possible values and saving them with probability proportional to their posterior probability. The strategy to sample these values is given by the MCMC algorithm, which comes in different flavours. I here briefly describe the Metropolis Hastings (MH) algorithm, because it is simple and it is commonly used in phylogenetic analyses (**Papers III**, **V** and **VI**). However, in some papers other algorithms were used in combination with MH (e.g. Gibbs sampling in **Papers I**, **III**, **IV** and **VI** and reverse-jump in **Paper VI**).

After the Markov chain is initiated with some plausible parameter value(s), a new value is proposed. The proposed parameter values at each step are randomly drawn, and depend on the current state of the chain but not on previous states. To move across parameter space, the proposal must be accepted. This occurs with probability *R*, such that:

$$R = min\left[1, \frac{f(X|\theta\prime)}{f(X|\theta)} \cdot \frac{f(\theta\prime)}{f(\theta)}\right] \tag{2}$$

The second element in the brackets is the ratio between the probability of the data, given the proposed parameter value θ′, and the probability of the data given the current parameter value θ (i.e. the likelihood ratio), multiplied by the ratio between proposed and current prior probabilities for the same two values. It can be seen by comparing (1) and (2) that this is equivalent to a ratio of posterior probabilities, as the marginal likelihood of the data remains the same for the two parameter values. Thus, if the proposed step increases the posterior probability (the right element is greater than 1) the move is always accepted. If the proposed step decreases the posterior probability, it might still be accepted, but the probability of acceptance is equal to this ratio.

The take home message is that the posterior probability can be described for statistical models of arbitrary complexity, without having to worry about the intractable marginal likelihood, as long as the chain has sufficiently explored the parameter space and the data are informative. This underscores the two properties of Bayesian inference that I think are important for the analyses in this thesis. First, Bayesian inference can be a powerful tool to estimate parameters in models of high complexity, for example phylogenetic models (**Papers III**, **V** and **VI**) and non-gaussian generalised mixed-effect models (Papers **I - IV** and **VI**). Here, MCMC is a more accurate approximation of the posterior probability than the approximations of maximum likelihood in restricted maximum likelihood estimation procedures. Also, the measures of uncertainty from Bayesian inference are more intuitive and can be carried on for subsequent variable transformations. Second, Bayesian inference forces us to be explicit about our assumptions of the underlying processes that generate observations (i.e. the priors), and also allow us to assess the sensitivity of our inference to those assumptions.

I used mixed-effect Bayesian models fitted by MCMC throughout this thesis. For example, I used them to jointly infer the prevalence and intensity of water-mite infections in natural populations of *I. elegans*, and to infer the reaction norm of fecundity in response to parasites for two of the female morphs (**Paper I**). I also used these models to estimate morph frequencies before and after colour development and hence infer pre-reproductive selection in these natural populations (**Paper IV**). Finally, I used mixed models in which phylogenetic relationships are treated as random effects, to investigate the ecological and demographic correlates of the presence of female-limited colour morphs across Coenagrionoidea (**Paper VI**). My phylogenetic analyses, further described in the following section, were also based on this inference framework.

Phylogenetic inference: graphical models and biogeographic dating

I used probabilistic acyclic graphical models in RevBayes (Höhna et al. 2016) for phylogenetic inference in **Papers III**, **V** and **VI** of this thesis. Graphical models provide a framework to represent the conditional structure of (usually complex) statistical models. The variables in a model are represented as the vertices (nodes) in the graph and the edges between them define their conditional relationships. In RevBayes, the user can specify the edges and nodes of any probabilistic model using the Rev programming language, as long as the desired distributions and functions that constitute the nodes are available.

A phylogenetic analysis typically consists of two parts, a model for the tree (e.g. Yule, birth-death, uniform), defining the branching structure (i.e. topology) and branch lengths, and a continuous-time Markov chain (CTMC) model describing the rate(s) of molecular evolution along the tree branches. Imagine a simplified case in which the tree (Ψ) is known with absolute certainty and we only wish to model molecular sequence evolution (S) according to an HKY substitution rate model. This model can be graphically represented as shown in Figure 11.

Here, the observed sequences (S) are an outcome of a PhyloCTMC process with parameters Ψ and Q. In this simplified example, Ψ is a single observation, maybe derived from alternative data. Q is the substitution rate matrix, that is, the matrix defining the relative rate at which alternative substitutions (e.g. A→T or C→G) occur in the sequenced region. Under the HKY model Q is a deterministic product of the stochastic parameters κ and π, which respectively represent the rate of transitions to transversions (two types of nucleotide substitutions) and the stationary nucleotide frequencies. As stochastic variables, κ and π are drawn from prior distributions (lognormal and Dirichlet) with constant parameter values, unless hyperpriors are specified. In the case of a partitioned analysis, each partition (e.g. gene or codon position) has its own Q κ and π.

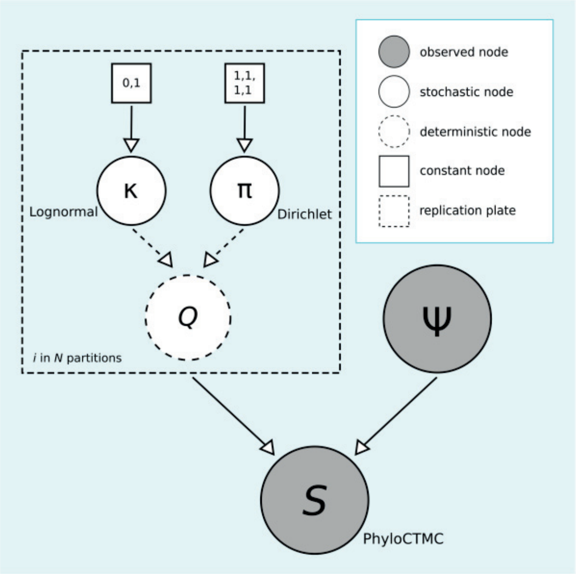
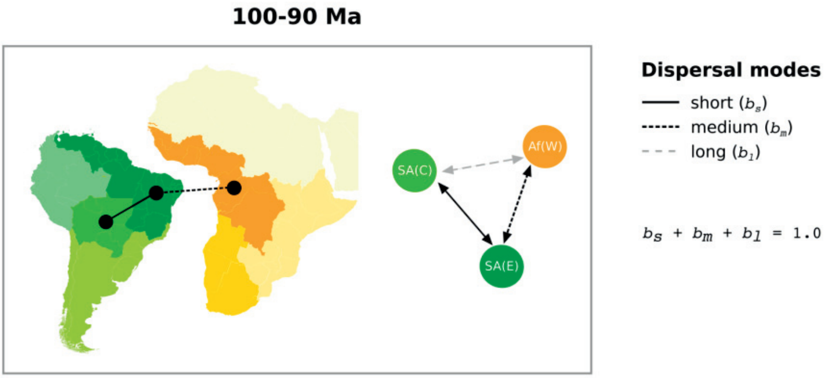


Figure 11. Graphical representation of a simple model of molecular evolution on a tree assuming HKY substitution parameters.

I used phylogenetic models specified in this fashion throughout this thesis, for example to infer the phylogeny of the genus *Ischnura* (**Paper III**) and to investigate trait-dependent diversification (**Papers V** and **VI**). Here, I further elaborate on the biogeographic model I used, following Landis (2017), to jointly infer speciation times and biogeographic history, across the entire Coenagrionoidea tree. Landis (2017) modelled dispersal probabilities as a weighted average of three dispersal modes: long-distance, medium-distance and short-distance dispersal (Fig. 12).

Empirical paleogeographic information is used to determine the presence of alternative dispersal paths during discrete time intervals since the oldest plausible age of the group. Ancestral ranges are therefore modelled as a time-heterogeneous CTMC. Due to the movement of continental land masses, the types of dispersal paths connecting areas change in a piecewise-constant manner through time. The main feature of this model is that alternative biogeographic and evolutionary scenarios are jointly considered while inferring speciation times. Therefore, molecular and biogeographic data of extant taxa together induce a posterior distribution of time-calibrated trees. The main modules of this graphical model are shown in Figure 13.



e.g.

$b_s = 0.7$
$b_m = 0.2$
$b_l = 0.1$

Dispersal rates q_{ij}

$q_{SA(E),SA(C)} = 0.7 + 0.2 + 0.1 = 1.0$
$q_{SA(E),Af(W)} = 0.2 + 0.1 = 0.3$
$q_{SA(C),Af(W)} = 0.1 = 0.1$

Mode-weighted matrix Q

$$Q = \begin{bmatrix} - & 1.0 & 0.3 \\ 1.0 & - & 0.1 \\ 0.3 & 0.1 & - \end{bmatrix}$$

Figure 12. Toy example of model-based dispersal and ancestral range inference, following Landis (2017). Dispersal paths are documented for each of a series of discrete time intervals. In this example, consider an interval between 100-90 Ma and a clade distributed across Western Africa (Af(W)), Eastern South America (SA(E)) and Central South America (SA(C)). During this period the areas SA(E) and SA(C) were linked by short-distance dispersal paths as there were no water barriers between them. Also in this epoch, SA had only started to drift away from Africa, hence SA(E) and Af(W) were connected by medium-distance dispersal. Af(W) and SA(C) were even further apart and could only be reached by long distance dispersal. The weighing parameters (b_i) of the three dispersal modes add to one. The lower panel depicts an example of one iteration in the Markov chain. The dispersal rates between geographic areas for this iteration are given by the sum of the appropriate weighing parameters. The matrix *Q* of dispersal rates is analogous to the substitution rate matrix of the molecular evolution model. In fact, in the full model, biogeographic ranges and molecular sequences jointly evolve along the tree branches.

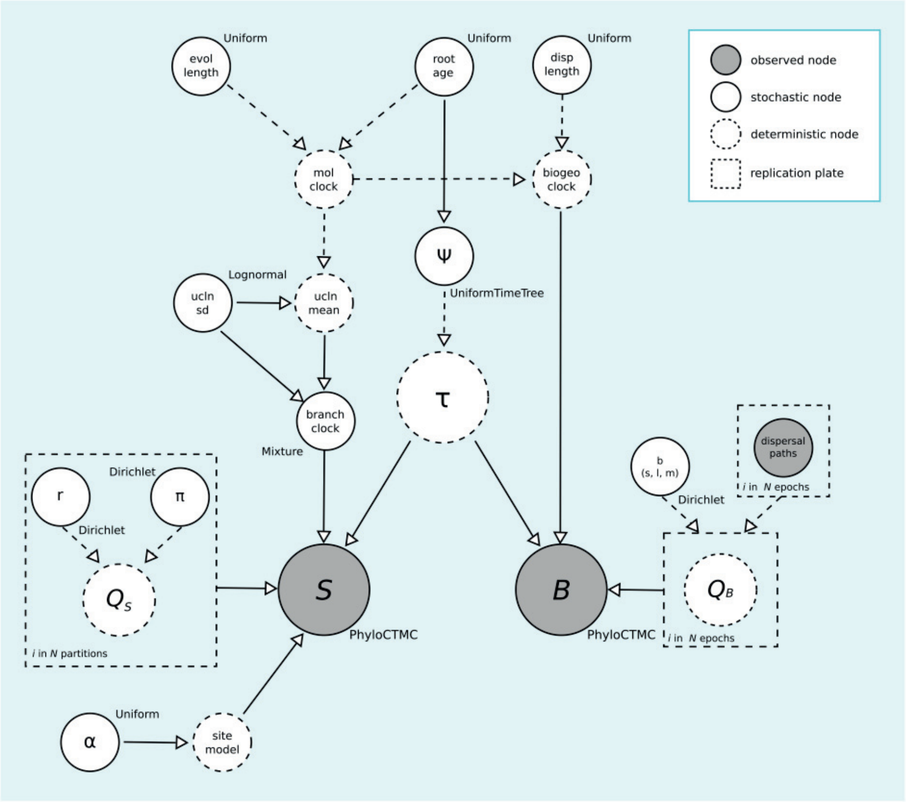


Figure 13. Graphical representation of the biogeographic dating model implemented by Landis (2017), and used in this thesis to jointly infer speciation times and ancestral ranges. For simplicity, I have omitted the constant nodes for the prior parameter values. These can be found in the Extended methods section of **Paper V**. Both the biogeographic (*B*) and molecular sequence (*S*) data are modelled with a CTMC process. Each CTMC process takes a clock, tree and *Q* parameter. Of course, both molecular sequences and biogeographic histories evolve along the same root-scaled species tree τ. The estimation of Q_B is described in Figure 13. The estimation of Q_S is similar as the one shown in Figure 12, except here the exchangeability rates are defined by a generalized time reversible model (GTR), which means that a different transition rate is inferred for each unique pair of nucleotides. The clock parameters depend on the total length of the tree (in molecular-evolutionary or dispersal units) and the root age. For the molecular model, evolutionary rates differ between branches, and this clock determines the mean of the the lognormal distribution of branch rates. The branch rates and number of rate categories are used to calculate the branch-specific clocks used by the molecular CTMC. The molecular CTMC also considers gamma-distributed among-site heterogeneity, with shape and scale parameters equal to α.

Results and Discussion

Ecological morph differences

Damselfly colour morphs are known to differ in several phenotypic traits, such as fecundity (Svensson and Abbott 2005), physical resistance to coercive male mating attempts (Gosden and Svensson 2009), morphology (Abbott and Gosden 2009), larval developmental time (Abbott and Svensson 2005), and thermal physiology and acclimation (Lancaster et al. 2017). My results add to this growing evidence of pleiotropic effects of the morph-determining locus, and highlight morph differences in ecologically relevant traits. In **Paper I**, I found that the two common female morphs of *I. elegans* in Southern Sweden differ in their defence mechanisms against parasitic water mites. If parasitized, A-females suffered on average greater infection intensities than I-females, yet their fecundity was relatively insensitive to mite infections (Fig. 14). While this higher fecundity tolerance of A-females might provide them with an advantage in environments where these parasites thrive, I-females are at an advantage in more benevolent environments due to their higher baseline fecundity (intercept difference in Fig. 14b).

These results are suggestive of correlational selection acting on combinations of defence traits that have redundant effects on fitness, as the two fundamentally different defence mechanisms were combined in different ways in the most common female morphs. This study also provides a complementary perspective to the role of NFDS maintaining female morphs locally. While current evidence strongly suggests that NFDS due to sexual conflict and male pre-mating harassment is sufficient to prevent morph fixation within populations (Le Rouzic et al. 2015), differences in defence traits may account, in part, for variation in morph frequencies among populations at a regional scale, if other factors (e.g. alternative hosts) influence geographic variation in parasite prevalence.

The female morphs in *I. elegans* also differ in the duration and temperature sensitivity of their post-emergence colour development. In **Paper IV**, we found that A-females, which do not deposit melanin on coloured areas during adult development, reach their final sexually mature colouration faster than I- and O-females. A-female survival through this developmental period is also less sensitive to temperature, whereas I- females, and to a lesser degree O- females, are more likely to successfully reach sexual maturity at a relatively higher ambient temperature (Fig. 15a).

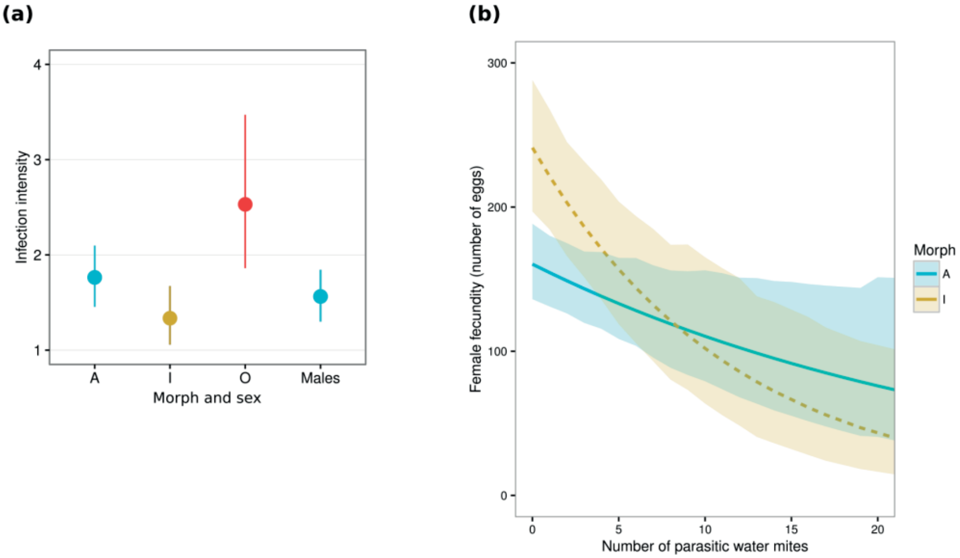


Figure 14. Morph defences against parasitic water mites in *I. elegans*. Female morphs differ in **(a)** resistance, measured as the infection intensity of parasitized individuals and **(b)** tolerance, measured as the reaction norm of fecundity to infection intensity. I-females had significantly higher resistance and lower fecundity tolerance to mite infections than A-females. O-females were overall rare in the study populations (~5%) and although their infection estimate has high uncertainty, they seem to be the most parasitized female morph. Circles and whiskers in **(a)** and lines and shaded areas in **(b)** show posterior means and 95% credible intervals.

In Southern Sweden, this difference results in higher adult pre-reproductive survival of A-females compared to the other two morphs. Pre-reproductive natural selection thus influences morph frequencies among individuals that will potentially be subject to frequency-dependence male harassment. Moreover, geographic variation in pre-reproductive selection might contribute to the latitudinal cline of A-female frequency observed across Europe. *I. elegans* is distributed throughout Europe and the Palearctic, with populations in Southern Sweden being close to the northern limit of the species range (Boudot and Kalkman 2015). These northern populations also harbour the highest frequencies of A-females across Europe (Fig. 15b), presumably due to an advantage of A-females, which have shorter developmental time and higher survival in cooler conditions. A recent study showing that A-females are more tolerant to cold shocks than I-females also supports this interpretation (Lancaster et al. 2017).

A consequence of this morph difference in pre-reproductive survival is that females may experience opposing selection pressures throughout their lives, with viability selection favouring A-females early in life and NFDS acting against them when sexually mature. The efficacy of any mimicry signal is expected to break down with increasing frequency of the mimic (Iserbyt et al. 2011; Finkbeiner et al. 2018). Thus, our results also suggest that high pre-reproductive survival of A-females at higher latitudes might cause stronger selection against these male-mimics via male harassment later in life. However, these frequency-dependent effects may be undone in the next bout of frequency-independent pre-reproductive selection. Morph differences in both defence mechanisms and developmental sensitivities show that other ecological factors than mating interactions and sexual conflict can influence the evolutionary dynamics of these polymorphisms, and underscore the importance of studying these processes in natural populations.

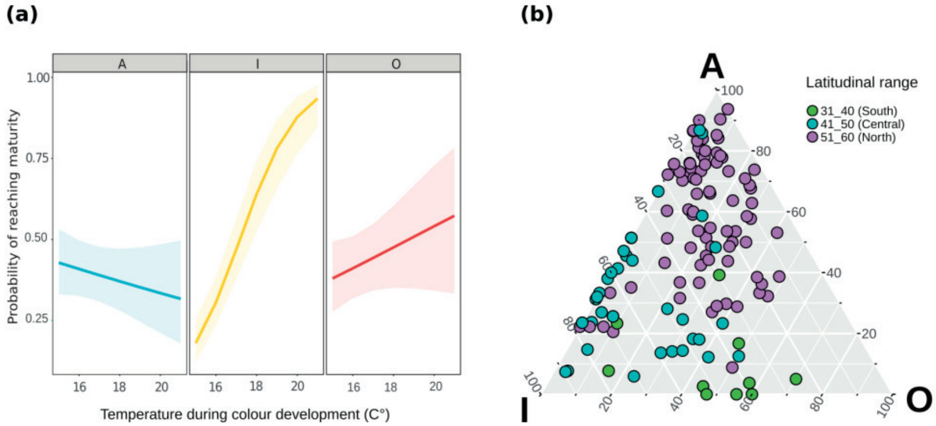


Figure 15. Temperature effects on developmental success and pre-reproductive survival **(a)** and latitudinal morph distribution **(b)** in *I. elegans.* Lines and shaded areas show mean estimates and 95% confidence intervals in **(a)**. Each circle in **(b)** corresponds to a European population. Each position in the ternary plot gives a specific combination of female-morph frequencies, with hypothetical monomorphic populations at the vertices in three latitudinal bands.

A glimpse into morph wiring

In **Paper II** I aimed to investigate if morph differences in adult developmental trajectories could drive some of the pervasive phenotypic differentiation that characterises these morphs in their final, sexually mature colour phase. We know that a large fraction of phenotypic variation among animals arises as a product of differences in the regulation of developmental processes (Carroll 2008). Therefore, I investigated if female morphs in *I. elegans* differ in the temporal association between colour development and developmental changes in gene expression.

We categorised colour development in two windows, marked by distinguishable colour changes. We found that most regulatory differences between morphs occur relatively late, when the morphs acquire their final colour patterns and reach sexual maturity (Fig. 16). In contrast, thousands of genes are similarly downregulated in all three morphs during the early phase of colour change. These results suggest that a slowdown of core metabolic processes is a main feature of adult development in all three morphs, yet specific regulatory differences, particularly during late colour development, are important in determining the phenotypes of sexually mature individuals.

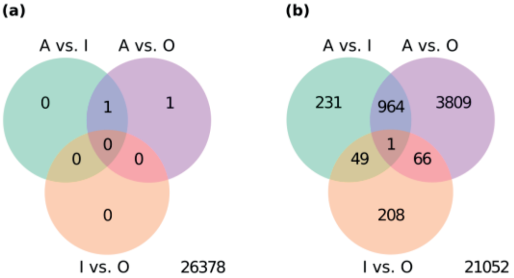


Figure 16. Venn diagrams of genes with significantly different developmental regulation between female morphs of *I. elegans*. Developmental regulation of gene expression was compared between morphs across **(a)** early and **(b)** late colour development. Most significant differences were detected during late colour development, when A-females were distinguished from both I- and O-females in the developmental expression changes of 965 genes.

Functional annotation of the *I. elegans* transcriptome showed that the observed regulatory patterns associated with the colour-morph locus may be related to some of the extensively recorded phenotypic differences between morphs. First, it is important to note that A-females acquire their final colour pattern at a younger age than I- and O-females, and available evidence indicates that these mature-coloured A-females are as likely to be capable of reproduction as older mature-coloured I- and O-females (**Paper II**). A-females differed from both I- and O-females in the developmental regulation of several genes with putative roles in reproductive physiology during late colour development, when they are younger, in absolute terms, than I- and O- females. These genes include regulators of the ecdysteroid hormone 20E, which is crucial for reproductive development, vitellogenin genes, and other genes associated with lipid transport to developing eggs. Thus both transcriptomic and phenotypic data provide some evidence of fast fecundity development in A-females, which may come at a cost in total fecundity, as revealed by the higher baseline fecundity in I-females, in **Paper I**.

A-females also seemed to have a more consistent pattern of upregulation of some pro-phenoloxidase activating factors during the late colour-development window. In insects, innate immune responses to extracellular pathogens, such as the water mites mentioned above, depend on the activity of phenoloxidases (Gillespie et al. 1997). Therefore, these developmental morph differences in the regulation of innate immunity may be associated with the differences in defence mechanisms highlighted in **Paper I**. However, as it often the case with immunity responses, I am currently unaware of whether these putative developmental differences in immunity regulation between morphs are a cause or a consequence of differential interactions with parasites.

Finally, *Dmrt*, a Doublesex and mab3-related transcription factor, was among the genes that showed a unique developmental regulatory pattern in A-females, compared to both I- and O-females. This is of particular interest because genes in this family have been associated with sex determination and sexual dimorphism in a variety of animals (Kopp 2012). In other insects, including one congeneric damselfly species (*Ischnura senegalensis*), alternative splicing and differential expression of *Doublesex* isoforms underlie female-limited colour polymorphisms (Kunte et al. 2014; Takahashi et al. 2018). It would be very interesting for future studies to investigate if the elusive colour-morph locus in *I. elegans* is a top regulatory gene, governing a pleiotropic developmental cascade via tissue-specific expression of *Doublesex*.

The descent of damselflies

As mentioned at the beginning of the Introduction, the classic explanations for sex-limited diversity in colour patterns come from a wealth of theoretical and empirical studies looking at male colour signals under sexual selection by female choice. It is therefore reasonable to ask if *Ischnura* is just an heretic lineage, or if instead, female-morph differentiation is a general evolutionary outcome of intense sexual conflict over mating, and it happens to involve colour traits in damselflies as these organisms are strongly visually driven. There are over 1 800 species of Coenagrionoidea, so investigating the evolutionary origin of female-limited polymorphisms in this superfamily would be a good starting place. Addressing this question, however, demands a phylogenetic inference framework, which has been lacking for the more than 20 years since the early studies on this system.

I inferred a multi-locus phylogeny of Coenagrionoidea using sequence data from 669 species worldwide (Fig. 17). In addition to providing a phylogenetic framework for addressing questions about phenotypic evolution, this study (**Paper V**) also clarified the macroevolutionary and biogeographic history of pond damselflies and their relatives the feather legs. These damselflies, like many other animal clades, have higher species richness in the tropics than in temperate areas. I found that this regional diversity pattern is a product of an early burst of diversification and a longer history of pond damselflies in the tropics.

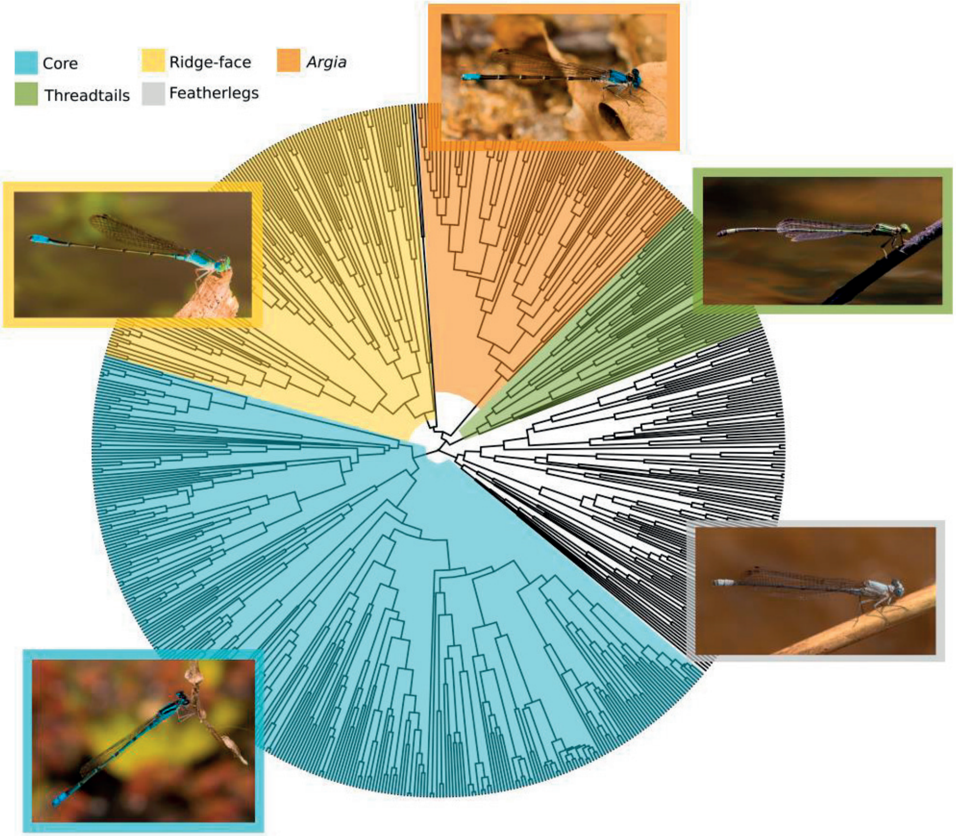


Figure 17. Phylogeny of pond damselflies and their relatives the featherlegs (superfamily Coenagrionoidea). The main clades are highlighted in different colours. A representative species in each clade is illustrated. Photo credits: E.I.S. and Erland Refling Nielsen (Threadtails).

After originating in the tropics, around 39 Ma, and rapidly acquiring a pantropical distribution, phylogenetic diversification in Coenagrionoidea has slowed down towards the present (Fig. 18). Lineages that dispersed to temperate areas speciate faster but also go extinct at a higher rate (Fig. 19). There is growing evidence that climatic fluctuations, which predominantly affect temperate areas, have caused species to be more ephemeral at higher latitudes (Weir and Schluter 2007; Botero et al. 2014). Thus, while the tropics continuously pump species into the comparatively depauperate temperate region (Fig. 18), the regionally lower diversity in the north does not seem to translate into higher diversification rates.

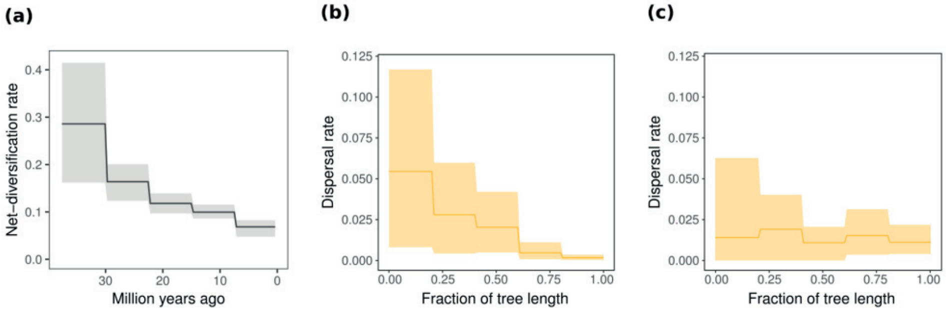


Figure 18. Temporal patterns of diversification **(a)** and dispersal **(b-c)** in the evolutionary history of Coenagrionoidea. Lines and shaded areas correspond to posterior means and 95% highest posterior density intervals. **(a)** Diversification rates decreased over time since the most recent common ancestor of all Coenagrionoidea. **(b)** Transoceanic dispersal among tropical areas also decreased over time, whereas **(c)** dispersal from the tropics to temperate areas has remained relatively constant. The analysis in **(b-c)** considered uncertainty in speciation times, and therefore the x axis is given in proportion to the total tree length rather than in absolute time.

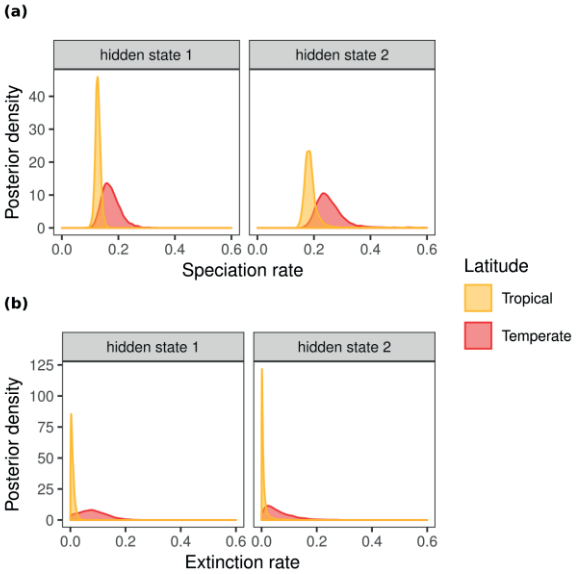


Figure 19. Diversification rates of tropical and temperate lineages of Coenagrionoidea. **(a)** Speciation and **(b)** extinction rates were estimated using a hidden state-dependent speciation and extinction model (Beaulieu and O'meara 2016), thereby accounting for background rate heterogeneity. This was done by modelling background rate heterogeneity as two states of a hidden trait and estimating latitude-dependent diversification rates for each of these rates.

These results are relevant to the classic discussion about the ultimate drivers of global diversity patterns that can be traced back to Darwin (1859) and Wallace (1878). For over a century, researchers have proposed numerous hypotheses to explain Earth's latitudinal diversity gradient (LDG). Yet, the discussion is far from settled, with some studies emphasizing historical contingencies (e.g. (Economo et al. 2018), and others pointing at ecologically driven diversification (Pyron and Wiens 2013). This study suggests that the longer tropical history in Coenagrionoidea may be responsible for the formation of the LDG in this group. However, the fluctuating climatic conditions in the temperate regions might accelerate lineage turnover, thereby contributing to the maintenance of the Coenagrionoidea LDG, in spite of continuous dispersal out of the tropics.

Evolution of female colour variation

With this phylogenetic framework at hand, I investigated the macroevolution of two female-limited forms of colour variation. First, within the genus *Ischnura*, I studied the evolution of male-like colour signals in immature females, as a means to reduce male pre-mating harassment (**Paper III**). Phenotypic manipulations of colour signals in *I. elegans* indicated that the expression of a blue abdomen patch, that is shared by males and immature females of all three morphs, reduces male approaches, especially towards female morphs that become melanized and more sexually dimorphic with reproductive development (Fig. 20).

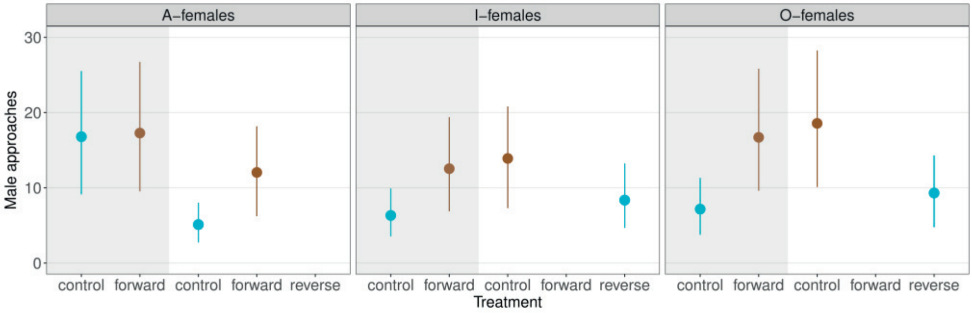


Figure 20. Male approaches to manipulated females displaying alternative colour development phenotypes. A male was presented with two females of the same morph and actual developmental stage. Results of trials with immature females as shaded in grey and trials with sexually mature females have a white background. Immature females were painted with their actual abdomen colour (control), or manipulated to advance their apparent developmental state (forward). Mature I- and O-females were manipulated to display their previous immature colour signal (reverse) or with their actual colour (control). Because A-females do not change the colour of their abdomen patch during adult development, mature A-females were manipulated to investigate how losing their blue colour would influence male approaches (forward). Circles and whiskers represent posterior means and 95% highest posterior density intervals, respectively.

This led us to hypothesize that male-mimicking A-females, could have evolved the continuous expression of this colour signal throughout their entire adult life, via the retention of an immature trait (neoteny), which already had a function preventing male harassment prior to the development of reproductive capacity. However, when I examined the phylogenetic distribution of this colour patch across females and males of *Ischnura*, I found no evidence that such a permanent expression of the blue patch was derived from ancestors in which the signal was expressed plastically over development. Instead, we were surprised to find that it is the more sexually dimorphic female morphs that have repeatedly evolved this signal, but always as a developmentally plastic trait and only among polymorphic lineages, where male-mimics are already present (Fig. 21). These results suggest that an anti-harassment colour signal, ancestrally present in males (Beatty et al. 2015) and presumably in male-mimicking females, has been repeatedly co-opted by sexually dimorphic female morphs to reduce harmful male harassment prior to reproductive maturity.

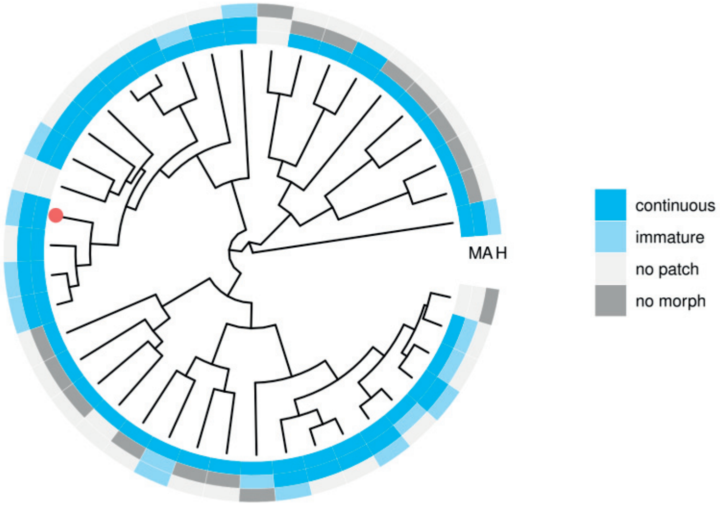


Figure 21. Phylogeny of the genus *Ischnura* and abdomen colour phenotypes in males (M) and female morphs (A and H). Female morphs across species were categorised as male-coloured (A) or sexually dimorphic (H) at sexual maturity. Within each morph, and for males, the expression of a blue abdomen patch could be continuous, if the patch is expressed throughout adult life, restricted to the sexually immature stage (immature) or absent (no patch). Some species are monomorphic and in those cases the phenotype for the missing morph is coded as ‘no-morph’. The phylogenetic position of *I. elegans* is marked by a red circle.

Second, I investigated the evolutionary origin and maintenance of female-limited colour polymorphisms across the entire Coenagrionoidea, and their consequences for phylogenetic diversification (**Paper VI**). I expected female-limited polymorphisms, to arise in lineages characterised by intense sexual conflict over mating. Measuring the intensity of sexual conflict directly in hundreds of taxa would be impractical, if at all possible (Arnqvist and Rowe 2002), but I could use demographic and ecological conditions that can be reasonably expected to promote such antagonistic inter-sexual interactions. Higher density of adult individuals at breeding sites is likely to lead to an increasing intensity of sexual conflict over mating, by increasing the overall rate of inter-sexual encounters (Martin and Hosken 2003; Levitan 2004). The density of breeding individuals might in turn depend on ecological factors, such as latitudinal distribution, which influences the length of the mating season, and habitat openness, which may restrict adult movement away from breeding sites.

I found that female polymorphisms have evolved repeatedly across Coenagrionoidea (Fig. 22), in association with temperate distribution ranges, open landscapes and high adult density at breeding sites (Fig. 22). Moreover, I found that polymorphisms typically evolve from sexually dimorphic ancestors, through the origin of a novel male-coloured female morph. This further suggests that female-limited colour polymorphisms emerge as a result of sexual selection by sexual conflict over mating. If these novels female morphs evolve by co-opting male-like and tissue-specific expression patterns of top regulator loci such as *Doublesex* (see **Paper II**), then this can help explain why male-mimicking morphs are so widespread, even though the specific colour patterns of males can be markedly different among species.

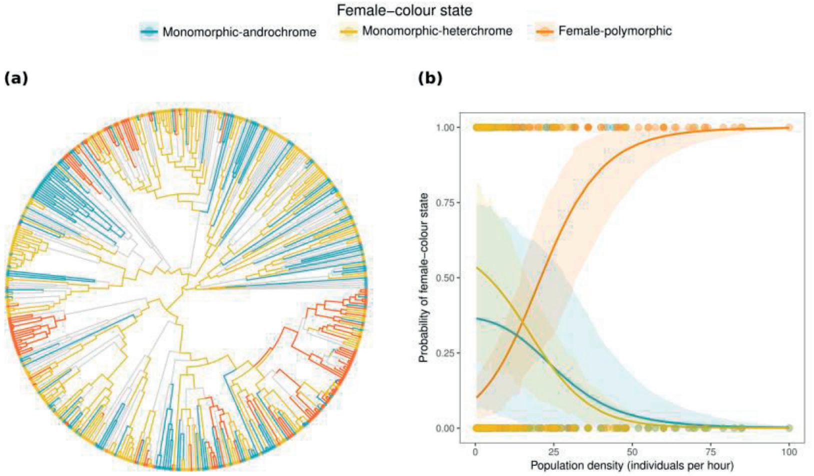


Figure 22. The origin of female-limited colour polymorphisms in pond damselflies and their relatives the featherlegs (superfamily Coenagrionoidea). Females in monomorphic lineages are either ‘androchrome’ if males and females have similar colour patterns or ‘heterochrome’ if they are sexually dimorphic. **(a)** Phylogeny of Coenagrionoidea showing female-colour states at the tips and reconstructed ancestral states. Branches with over 5% uncertainty in their female-colour state are in grey. **(b)** Effect of density at breeding sites on the probability of female-colour states. The lines and

shaded areas represent posterior mean effects and 95% highest posterior density intervals. The circles correspond to natural populations of 79 species surveyed in the field.

Finally, I gained some insights on the fate of female-polymorphic lineages. I found that polymorphic lineages have a remarkably high turnover rate, they 'speciate faster and die younger' than female-monomorphic species (Fig. 23). This does not seem to be merely a consequence of a majority of female-polymorphic taxa having a temperate distribution (compare x axes of Fig. 19 and 23). Instead, these results indicate that sexual conflict can have dramatically different effects at different time scales, promoting female-specific diversification within species and driving fast extinction at a macroevolutionary scale.

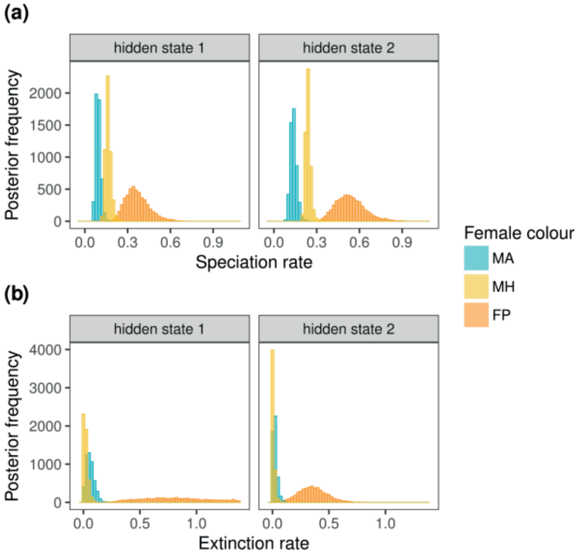


Figure 23. Diversification rates in lineages with alternative female colour states. There are three possible female colour states: monomorphic-androchrome (MA), if males and females have similar colour patterns, monomorphic-heterochrome (MH) if they are sexually dimorphic and female-polymorphic (FP). **(a)** Speciation and **(b)** extiction rates were estimated using a hidden state-dependent speciation and extinction model (Beaulieu and O'meara 2016), thereby accounting for background rate heterogeneity. This was done by modelling background rate heterogeneity as two states of a hidden trait and estimating latitude-dependent diversification rates for each of these rates. The histograms represent posterior distributions of rate estimates.

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